

Analysis of Chromosome Abnormalities in Human Oocytes and Embryos

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Abbreviations:

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aCGH	Microarray comparative genomic hybridization
AMA	Advanced maternal age
Bla	Blastomere
CR	Chromosome rearrangement
CGH	Comparative genomic hybridization
der	Derivative
DOP-PCR	Degenerate oligonecleotide primed PCR
FISH	Fluorescent *in situ* hybridization
ICE	Inter-chromosomal effect
ICM	Inner cell mass
IVF	*In vitro* fertilization
PB	Polar body
PBS	Phosphate-buffered saline
PGS	Preimplantation genetic screening
PGD	Preimplantation genetic diagnosis
RCT	Randomized clinical trial
RM	Recurrent miscarriages
RIF	Repeated implantation failure
SNP	Single nucleotide polymorphism
SSC	Saline-Sodium Citrate
TBE	Tris/Borate/EDTA
TE	Trophectoderm cells
WGA	Whole genome amplification

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Abstract

Title: Analysis of chromosomal abnormalities in human oocytes and embryos.

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The chromosome constitution of human cleavage stage embryos has been extensively investigated using a variety of techniques, revealing high levels of aneuploidy and mosaicism. However, the final phase of preimplantation development, the blastocyst stage has received relatively little attention mostly because it is only recently that embryo culture has become sufficiently well optimised to reliabley generate blastocysts. One of the aims of this study was to examine blastocyst cytogenetics, characterising the extent and variety of aneuploidy and, where possible, determining the origin of the abnormalities detected. Both the frequency of aneuploidy and the incidence of mosaicism were significantly lower in the 52 embryos generated by 20 patients that had successfully undergone the first cellular differentiation, producing trophectoderm (TE) and inner cell mass (ICM). Valuable tools for the detailed chromosomal analysis of blastocysts, used in both research and clinical contexts, were comparative genomic hybridization (CGH) and array CGH (aCGH). However, validation of these methods, especially aCGH, was required in order to verify accuracy. A low error rate and a low misdiagnosis risk were demonstrated.

The morphology of 1397 embryos at the cleavage and blastocyst stages from 229 patients was evaluated in relation to their chromosomal complement. The results obtained during this part of the project showed that, in general, there is little correlation between cleavage stage morphology and chromosome status. A weak link between morphology and aneuploidy, however, was found for embryos at the blastocyst stage. Chromosomally normal female embryos had a tendency to grow faster than male embryos at the cleavage stage and therefore tended to achieve superior morphological scores, whereas the trend was reversed at the blastocyst stage. Abnormal embryos carrying types of aneuploidy compatible with formation of a clinically recognised pregnancy had morphologies indistinguishable from those of euploid embryos.

This study also aimed to utilise aCGH for the preimplantation genetic diagnosis (PGD) of imbalances due to structural chromosome rearrangements (e.g. translocations) in 39 carriers, a total of 139 embryos were assessed. The data obtained revealed that carriers of Robertsonian translocations are at increased risk of aneuploidy affecting additional chromosomes not involved the translocation, a phenomenon known as an interchromosomal effect (ICE). Finally, the clinical outcomes of 300 patients undergoing preimplantation genetic screening (PGS) using aCGH, for various different indications, were evaluated at both the cleavage (795 embryos) and blastocyst stages (1097 embryos). The pregnancy rate following cleavage stage biopsy was significantly lower than following blastocyst stage biopsy. The miscarriage rate was significantly reduced following PGS for patients with recurrent miscarriages.

This work provided promising data supporting the clinical use of comprehensive chromosome analysis for the screening or diagnosis of preimplantation embryos and also yielded scientifically useful information concerning the frequency and nature of aneuploidy at the final stage of development before implantation.

1. Introduction

1.1 Human reproductive capacity and infertility

Humans as a species are not as fertile as most mammals for which data is available, as evidenced by the fact that the chances of conception are only around 25% per menstrual cycle (Bonde et al., 1998). Moreover, it has been estimated that 25% of women trying to achieve a pregnancy suffer from at least one spontaneous abortion and almost half of all implantations are lost before birth (reviewed in Warren and Silver, 2008). In 2009, the World Health Organization (WHO) for the first time characterised infertility as a "disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse" (Zegers-Hochschild et al., 2009). It is estimated that infertility affects one in seven couples (10-15%) worldwide (Boivin et al., 2007; Ombelet et al., 2008).

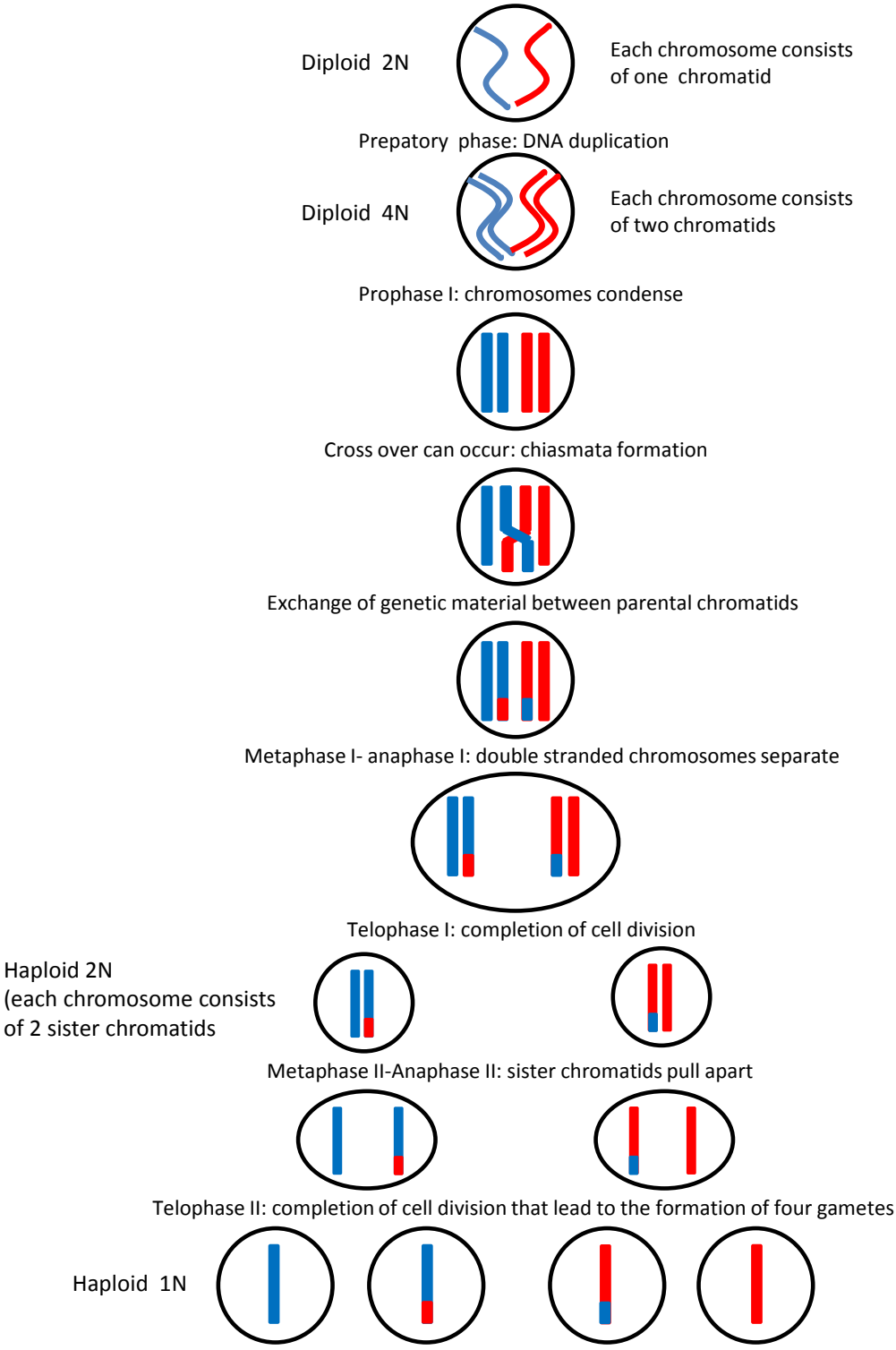
Infertility can affect both males and females. Conditions such as ovulatory failure, tubal damage, endometriosis, mucus defects/dysfunction, sperm dysfunction, antisperm antibodies, can all lead to the inability to achieve and/or maintain a clinical pregnancy (reviewed in Hull, 1990).

1.2 Gametogenesis

Formation of the human gamete takes place via a specialized cellular division which occurs only in reproductive organs, i.e. ovaries in the female and testis in the male. This specialised division is called meiosis and results in the reduction of the diploid (2N, 46) chromosome complement to haploid (N, 23) in the mature gamete. Meiosis consists of two separate divisions, meiosis I and meiosis II. Before the cell enters meiosis, during interphase, DNA duplication takes place, after which each parental chromosome consists of two sister chromatids held together at the centromere and by molecules of the cohesin protein complex (Parisi et al., 1999; Watanabe and Nurse, 1999; reviewed in Sakuno and Watanabe, 2009). At the end of meiosis I, two daughter cells are formed each containing one copy of each chromosome, which in turn consists of 2 sister

chromatids (reductional division). During meiosis I, crossover between the two parental homologous chromosomes takes place and results in the exchange of chromosomal material. Crossover points are known as chiasmata and they play an important role in the cohesion between the homologous chromosomes, especially during female meiosis (oogenesis) (reviewed in Handyside, 2012). During meiosis II the two sister chromatids separate (equatorial division) and this results in the formation of 4 haploid cells (Figure 1-1).

Figure 1-1: Steps of meiotic division



Meiosis consists of 2 divisions, meiosis I and meiosis II. In the preparatory phase, chromosome duplication takes place and each chromosome consists of 2 sister chromatids. In the prophase of MI, the 46 chromosomes condense and form 23 pairs of homologous bivalents. Crossover follows and homologous chromosomes associate with each other through the formation of chiasmata. Chromosomes align on the meiotic spindle in metaphase I and each chromosome separates to a daughter cell during anaphase I and telophase I (reduction division). During metaphase II, chromosomes consisting of 2 chromatids align on the meiotic spindle and sister chromatids separate into daughter cells during anaphase II and telophase II.

1.2.1 Spermatogenesis

The production of spermatozoa takes place in the testis of the adult male, starting at puberty and continuing throughout a man's life. Spermatogenesis leads to the formation of four haploid cells, called round spermatids, which in turn go through different maturation stages (elongation, condensation of the chromatin, histamine substitution with protamines and the formation of the acrosomal region). At the end of this maturation period the mature spermatozoa are formed (reviewed in Johnson, 1995; Wouters et al., 1998).

Sub-fertility affects around one in fifteen men (Hull et al., 1985) and a male factor is thought to contribute to ~50% of all infertility cases (De Krester and Baker, 1999). The main causes of male infertility are idiopathic, undescended testis, endocrinologic abnormalities, obstruction of the seminal tract, inflammation (epideidymitis, prostatitis), and sexual disorders (ejaculatory failure, erectile disorders) (Namiki, 2000). Idiopathic male infertility is perhaps the most frequently observed, leading to approximately 50% of all male factor cases. Genetic factors are thought to be involved in a large proportion of idiopathic infertility cases (Lilford et al., 1994). Specifically, microdeletions affecting the long arm (q) of the Y-chromosome in the Azoospermic factor region (AZF) have been associated with azoospermia (absence of sperm in the ejaculate) or oligozoospermia (reduced sperm count in the ejaculate) (Choi et al., 2004) along with deletions in other gene families such as RBM (RNA-binding-motif) and DAZ (Elliot and Cooke, 1997). Moreover, phospholipaze C zeta (PLCζ) has been identified as the sperm-derived oocyte activation factor that plays an essential role in oocyte activation through mediating the release of calcium ions (Ca^{2+}) followed by (Ca^{2+}) oscillations (Saunders et al., 2002; Swann et al., 2004; Parrington et al., 2007; Yoon et al., 2008). PLCζ in human has been found to be exclusively expressed in testis (Saunders et al., 2002) and was found to be present in the testis of non-mammalian species such as chicken (Coward et al., 2005). In some cases, failure of oocyte activation after intracytoplasmic sperm injection (ICSI), which is mainly used for male factor infertility treatment (reviewed in Forti

and Krausz; 1998), has been linked to mutations affecting PLCζ gene (Heytens et al., 2009; Kashir et al., 2011; Kashir et al., 2012).

1.2.2 Oogenesis

Oogenesis in the female begins during early foetal life (6th week of development until the 20th week) when oogonia cells start a series of mitotic divisions (Pinkerton et al., 1961) that lead to the formation of around 7 million germ cells. These are known as the primary oocytes and are located in the primordial follicles which progress through meiosis I and arrest at the diplotene stage of prophase I until adolescence (Gilbert, 2000). The nucleus of these arrested primary oocytes enlarges and forms a cellular structure known as the germinal vesicle (GV) (Larsen, 2001). At birth, around 0.5 – 2 million immature oocytes are remaining and they continue to die after birth. Ultimately, only approximately 400 oocytes will mature and be ovulated during the female's lifetime (Larsen, 2001). After puberty and during each menstrual cycle, a few primordial follicles start to grow. Epithelial granulosa cells surrounding the immature oocyte change from flat to cuboidal (Gilbert, 2000; Larsen, 2001). In addition, the oocyte itself increases in diameter (from 10 µm in a primordial follicle to ~80 µm in the fully developed follicle) (Gilbert, 2000) and the follicle that contains this oocyte is now known as the primary follicle. A thin layer of glycoproteins, composed of zona pellucida glucoproteins (ZP1, ZP2, ZP3 and ZP4) (Shabanowitz and O'Rand, 1988; Lefievre et al., 2004), are secreted from the oocyte and the primary follicle, surround the oocyte and form the zona pellucida. During every cycle in the female, almost all growing follicles become atretic and only one follicle, along with the oocyte, matures.

During the menstrual cycle, a hormone secreted from the hypothalamus, known as gonadotrophin releasing hormone (GnRH), is responsible for the release of follicle stimulating hormone (FSH), initially, and later the luteinizing hormone (LH) from the anterior pituitary gland. Fol-

licular cells start to express receptors for FSH and LH (later on). A surge in LH on day 14 of the cycle leads to: 1) completion of MI with extrusion of the first polar body (PB); 2) initiation of MII which arrests in metaphase II; 3) maturation of the oocyte's cytoplasm, preparing it for sperm penetration, fertilisation and support of the first few embryonic divisions (Gilbert, 2000; Larsen, 2001).

Ovulation occurs when the mature follicle ruptures and the oocyte is released into the fallopian tube surrounded by the cumulus cells and the corona radiata. As a result of the LH surge, the remainder of the ruptured follicle forms the corpus luteum which secretes progesterone and estradiol, which has the effect of preparing the lining of the uterus (endometrium) for embryo implantation.

1.3 Fertilization and preimplantation embryo development

The oocyte is usually released into the fallopian tube around day 14 of the menstrual cycle. To achieve fertilisation spermatozoa must swim by the activity of the flagellum towards the oocyte. Studies have suggested that chemotaxis (detecting chemicals secreted from the oocyte and/or surrounding cells) is responsible for directing the sperm's movement (Eisenbach et al., 2006). During the sperm's journey within the female reproductive tract, it undergoes essential changes known as capacitation which involves removal of inhibitory factors (Wassarman, 1999) and prepares the sperm for the acrosomal reaction. As the sperm reaches the oocyte and penetrates the surrounding layers of cells, the egg-binding protein on the sperm, suggested to be the equatorial segment protein (ESP) (Wolkowicz et al., 2008) or a protein from the cystein-rich secretory proteins (CRISP) family (Cohen et al., 2001, 2008), interacts with the sperm receptor on the zona pellucida (glycoprotein ZP3) and this binding induces the acrosomal reaction. This leads to the secretion of enzymes stored in the acrosome of the sperm to enable zona perforation, fol-

lowed by sperm penetration into the perivitelline space of the oocyte and gamete fusion. Sperm penetration activates the oocyte which in turn, resumes and completes the second meiotic division, extruding the second polar body. Once the oocyte completes the second meiotic division, male and female pronuclei begin to move towards each other, the pronuclear membranes disintegrate, paternal and maternal chromosomes mix and the fertilized oocyte becomes a zygote. The spindle of the first mitotic division starts to form directed by the sperm centrosome (Sathananthan, 1998). Oocyte activation involves multiple other processes as well as resumption and completion of the second meiotic division, such as exocytosis of cortical granules, cell cycle progression and pronuclear formation. All these processes are essential for embryonic development. This activation requires repeated oscillations of Ca^{2+} in the cytoplasm as a direct result of inositol triphosphate (IP_3) mediated calcium release by the activity of sperm-factor PLCζ (reviewed in Kashir et al., 2010). On the other hand, Aarabi et al (2012) investigated the localization of PLCζ in human and mouse sperm and found that active forms reside in sperm fractions other than the preinucealar theca, where the sperm-factor necessary to activate the oocyte is expected to reside. PLCζ was thought to be expressed exclusively in the testis in human while Aarabi et al (2012) found that it is expressed in the epididyaml epithelial cells, which explains its presence in the sperm head. More importantly, PLCζ was not detectable after the acrosome reaction had occurred and therefore will not be incorporated into the oocyte cytoplasm (Aarabi et al, 2012).

Within 24 hours of fertilisation, the zygote embarks on a series of mitotic divisions, known as cleavage. The cells formed in this way are called blastomeres and continue to divide in an asynchronous manner. Blastomeres formed from the first two to three divisions are spherical and totipotent. This totipotency, however, is lost as preimplantation development progresses (Hansis and Edwards, 2003; reviewed in Hansis, 2006).

Embryonic cells communicate with each other through two types of junctions: gap junctions and desmosome-like structures. Gap junctions, containing protein connexin-43 (CX-43)

which enables the transfer of ions and small molecules between embryonic cells, start to appear at the 4 cell stage (Hardy et al., 1996) and increase in number as the embryo continues to divide and reaches the blastocyst stage (Lopata et al., 1982; Sathananthan et al., 1990; Dale et al., 1991). At this final stage before implantation, dense arrays of these junctions are found mostly between trophectoderm cells (TE) and less frequently between inner cell mass cells (ICM) (Hardy et al., 1996). Tight junctions, which seal the intercellular cavity from the surrounding environment of the embryo, are essential to the formation of the ICM and TE cells and start to appear from the six to eight cell stage onwards (Tesarik, 1989; Dale et al., 1991) while desmosomes are first observed at the 16-cell stage and are found among TE cells only (Hardy et al., 1996).

Embryonic genome activation and gene expression is initiated at the 4-8 cell stage. Until that time the cleaving embryo is dependent upon proteins and mRNA stored in the oocyte (Braude et al., 1988). Embryonic genome activation coincides with the degradation of the last maternal mRNA/proteins which cannot be detected beyond the 6-cell stage (Heikinheimo et al., 1995).

By day-3 post fertilisation, embryos typically consist of 6-10 blastomeres and are preparing to undergo compaction and the first cellular differentiation. Compaction usually starts around day-4 (16-32 cell stage), after which individual blastomeres become difficult to visualise using conventional microscopy. The embryo at this stage is referred to as a morula (Larsen et al., 2001). Blastomere flattening and polarization leads to an increase in adherence and maximisation of cellular communication. At the end of compaction the first cellular differentiation takes place in the embryo and two different cell types are formed: the inner cell mass (ICM) which will form the embryo proper and the trophectoderm (TE) layer from which the extra-embryonic tissues are derived (e.g. placenta). Cavitation of the embryo involves the accumulation of liquid among ICM cells and leads to the formation of the blastocoele cavity. An embryo five days after fertilisation of the oocyte, with differentiated ICM and TE cells and a blastocoel cavity, is referred to as a blas-

tocyst. It is at the blastocyst stage that a naturally conceived embryo would normally arrive in the uterus and attempt to implant. Two different hypotheses have been put forward to explain how a blastocyst is formed: the inside-outside hypothesis and the polarization hypothesis. The inside-outside hypothesis is based on the notion that blastomeres located within the morula will form the ICM while the external cells will form the TE layer (reviewed in Edwards and Beard, 1997). According to the polarization hypothesis, oocytes and cleavage stage embryos are polarised in terms of the distribution of certain proteins and mRNA species and subsequent cleavage divisions can amplify these pre-existing gradients. It has been suggested that cells that divide perpendicular to the axis of polarity will form one polarized external cell which will contribute to the TE layer and one internal apolar cell that will form the ICM (reviewed in Oron and Ivanova, 2012).

Once the blastocyst arrives to the uterus, it releases enzymes to help penetrate and open the zona pellucida in order for it to hatch. The blastocyst is now ready to initiate communication with the maternal tissue and interact with the endometrium in order to implant (Larsen et al., 2001).

1.4 Implantation

Implantation is a very complicated process which is essential for the embryo to gain access to maternal nutrients. In order for this process to be successful, bi-directional communication between the blastocyst and the endometrium is required and this process is likely to be mediated through signalling and adhesion molecules (Larsen et al., 2001). The identification of the specific molecules involved in this process has been problematic so far as sites of implantation in human are not accessable to experimental studies *in vivo* (Carver et al., 2003).

Previous studies have shown different levels of expression of specific molecules in the endometrium at different stages of the menstrual cycle which indicates their involvement in the

implantation process, such as the extracellular matrix receptor integrin $\alpha_v \beta_3$ (Lessey et al., 1992) , leukemia inhibitory factor (LIF) (Cullinan et al., 1996) and heparin-binding epidermal growth factor (HB-EGF) (Yoo et al., 1997). On the other hand, investigation of expression profiles of molecules in human blastocyst revealed the presence of different integrins (Campbell et al., 1995) and HB-EGF receprtor Erb B4 (Carver et al., 2003) suggesting that these molecules may have essential roles during implantation.

Different model systems have been suggested and studied to allow the proper understanding of the molecules involved in the implantation process such as co-culturing embryos at the blastocyst stage on a monolayer of human endometrial stromal cells (Carver et al., 2003). More recenetly 3D culture systems involving Matrigel have been developed and used for extracellular matrix/stromal cell invasion assays with human blastocysts (Mardon et al., 2007). Systems employing human plasma as a provider of fibrin matrix, along with endometrial stromal and epithelial cells, have also been used, mimicking the archticture of human endometrium (Wang et al., 2011).

1.5 Incidence of chromosome abnormalities in human reproductive material

Human chromosomes are grouped into seven groups (A to G) based on descending order in size and the positioning of the centromere according to the international system for human cytogenetic nomenclature (ISCN 2013) (Figure 1-2). These groups are as follows:

- Group A: includes chromosomes 1 to 3 which are large metacentric chromosomes that can be distinguished from each other by size and centromere position.
- Group B: includes chromosomes 4 and 5 which are large submetacentric chromosomes.

- Group C: includes chromosomes 6 to 12 and X. Chromosomes in this group are medium-sized and metacenteric or submetacentric.

- Group D: includes chromosomes 13 to 15. Medium-sized acrocentric chromosomes, i.e. the short (p) arm consists of satellites.

- Group E: includes chromosomes 16 to 18 which are relatively short metacentric or sub-metacentric chromosomes.

- Group F: includes chromosomes 19 and 20 which are short metacentric chromosomes.

- Group G: includes chromosomes 21, 22 and Y. These are short acrocentric chromosomes with satellites in the p arm (Y chromosome bears no satellites).

Figure 1-2: Classification of human chromosomes into seven groups (A to G) according to size and position of centromere

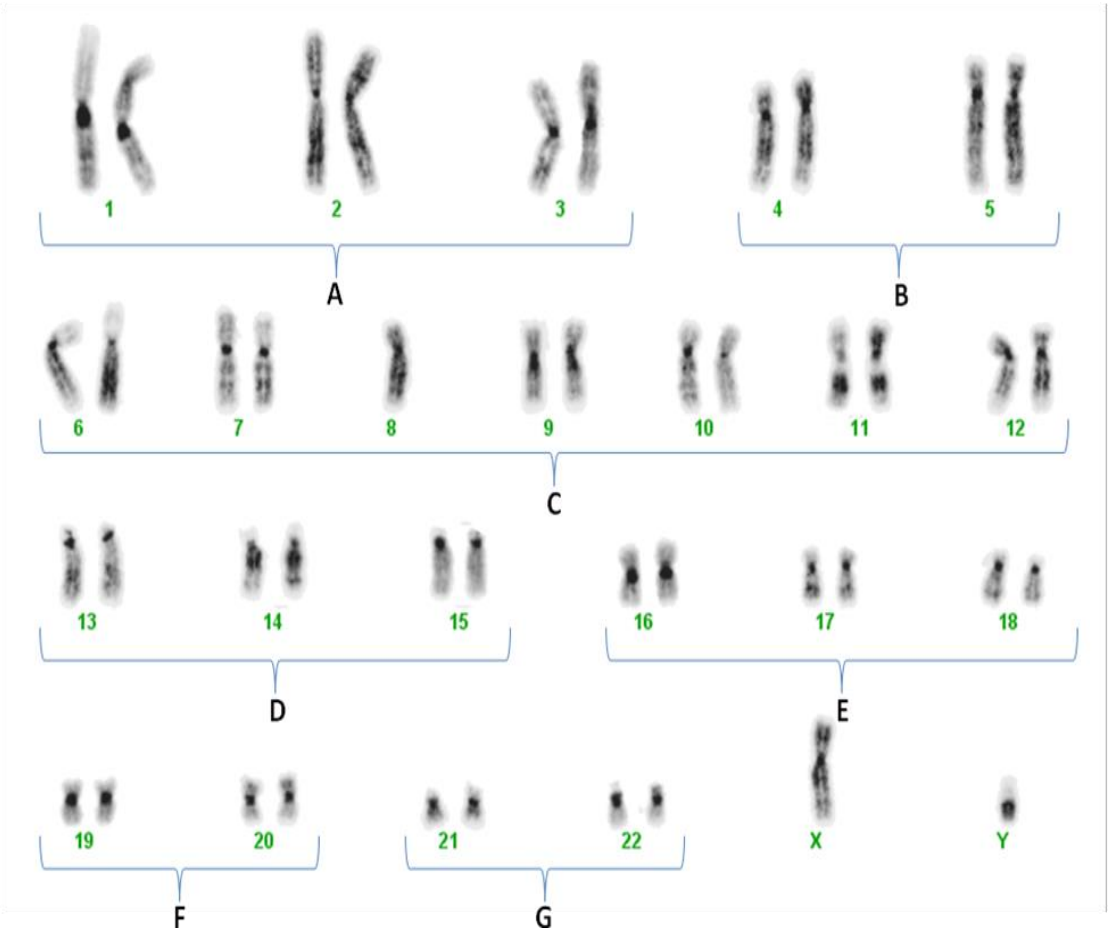


*Image courtesy of *Samer Alfarawati*

A relationship between chromosome abnormalities and miscarriages was initially suggested during the early sixties (Carr, 1963; Szulmann, 1965; Bowen and Lee, 1969). Many studies on a large number of samples obtained from products of conception followed and aneuploidy rates ranging between 32% and 60% were reported (Boue et al., 1975; Kajii et al., 1980; Warburton et al., 1979; Hassold et al., 1980; Lin et al., 1985; Dejmek et al., 1992). More recently, Menasha et al. (2005) in their retrospective study on spontaneous abortion cases between 1998 and 2002 including the analysis of 1273 specimens reported an overall aneuploidy rate of 65% for an average female age of 36.6 years. Furthermore, the frequency of chromosome abnormalities in live births was reported by Hassold and Jacobs (1984) to affect around 0.3% of newborns, while the estimated rate of aneuploidy in still births was approximately 4% (13-fold higher than in live births).

Cytogenetic abnormalities can be divided into those that affect the number of chromosomes (numerical) and those that involve alterations to the structure of chromosomes.

1.4.1 Numerical chromosome abnormalities

Numerical chromosome abnormalities are further subdivided into aneuploidy, which is the gain (e.g. trisomy) or loss (e.g. monosomy) of one or more chromosomes, and polyploidy; the gain of one or more whole sets of chromosomes (e.g. triploidy, 3N, tertraploidy 4N, etc).

Aneuploidy is the most common type of chromosome abnormality in humans and the most important clinically as it is considered the leading cause of mental retardation in the general population (Hassold et al., 1996). Trisomy 21 is the most frequently observed aneuploidy in live births, while trisomies 13 and 18 and XXY, XXX and XYY follow. As far as monosomies are concerned, the only one identified in live births is 45,X0 (Turner's syndrome). Trisomies and monosomies affecting other autosomes (apart from 13, 18, 21 and sex-chromosomes) are not seen in live births in non-mosaic form. Conversely, anomalies affecting all chromosomes have been de-

tected in material from spontaneous abortions, with trisomies being the most frequently seen type. Of these, trisomy 16 is the most common, followed by trisomies 21 and 22 (Menasha et al., 2005).

The advent of *in vitro* fertilization (IVF) provided unprecedented access to human embryos and gametes, especially oocytes, enabling detailed study of the "parental" mechanisms leading to aneuploidy. Furthermore, DNA polymorphism investigations, carried out on DNA from products of conception and children born with aneuploid syndromes, allowed researchers to determine the origin of aneuploidy; whether maternal or paternal and whether it occurred during meiosis I or meiosis II (Hassold et al., 1996). Data obtained during such investigations demonstrated that more than half of the abnormalities affecting the sex-chromosomes are of paternal origin, while the vast majority (90%) of autosomal trisomies are of maternal origin (Hassold et al., 1996). The main mechanisms leading to aneuploidy during meiosis are chromosome non-disjunction observed for males and females and during both MI and MII, and unbalanced premature separation of sister chromatids observed during female meiosis I. The latter of these two mechanisms is by far the most important source of aneuploidy in human oocytes (Gabriel et al., 2011; reviewed in Fragouli et al., 2011a).

Chromosome malsegregation errors can also arise during the post-fertilisation mitotic divisions in embryos throughout preimplantation development. The aneuploidy-causing mechanisms described post-zygotically are mitotic non-disjunction (reciprocal losses and gains of the affected chromosome in daughter cells), anaphase lag (chromosome loss in one or more of the daughter cells) and endoreduplication (chromosome gain in one or more of the daughter cells) (Coonen et al., 2004; Daphnis et al., 2005; Daphnis et al., 2008). These post-zygotic malsegregation events will lead to chromosomal mosaicism in the embryo, i.e. the presence of two or more cytogenetically distinct cell lines in the same embryo. Two main types of mosaic embryos have been categorized; mosaic diploid/aneuploid and mosaic aneuploid. In the mosaic aneuploid em-

bryo all the cells are chromosomally abnormal. It is thought that such embryos are usually derived from aneuploid zygotes (having chromosomal abnormalities of meiotic origin), which then undergo additional errors in mitotic chromosome segregation, producing aneuploidy cell lines with different abnormalities. However, in the mosaic diploid/aneuploid embryo some cells are chromosomally normal and a subset of cells were affected by a mitotic malsegreagation leading to chromosome abnormality(s).

Generally, in products of conception maternal MI errors are more common than MII errors. However, specific chromosomes had differences in non-disjunction patterns, for example trisomy 21 was predominantly an MI error and trisomy 18 was predominantly a result of MII error (Hassold et al., 2007). However, the comprehensive analysis of the entire chromosome complement of the 1st and 2nd polar bodies generated from 308 oocytes with an average female age of 40.8 years showed that the number of errors originating during MII was higher than that originating at MI (360 in MII vs. 229 in MI) (Fragouli et al., 2011b). Moreover, a clinical pilot study performed recently where PB1, PB2 and their corresponding oocytes were analysed (a total of 105 samples) reported that although the number of errors (125) in 1st polar body (meiosis I error) was higher than that observed (102 errors) in 2nd polar body (meiosis II error), the net number of errors observed in the oocyte were mostly of meiosis II origin as 48 of the meiosis I errors were combined with a balanced gain/loss in the 2nd polar body resulting in normal zygote (Handyside et al., 2012). It seems that the first meiotic division is the most prone to error in oocytes from younger women, as shown by the studies of products of conception mentioned above. However, the risk of meiosis II error increases dramatically with advancing female age, more rapidly than the increase in meiosis I errors, and ultimately become the dominant source of aneuploidy.

The correlation between advancing female age and increased aneuploidy rate has been long established (Angell et al., 1983; Delhanty et al., 1993; Munne et al., 1993; Iwarsson et al., 1999; Wilton et al., 2003; Fragouli et al., 2008; 2010; 2011c). Abnormalities in the structure of the

spindle (Battaglia et al., 1996), impaired function of the mitochondria (due to the accumulation of mutations with age) that affects cell cycle progression (Muller-Hocker et al., 1996; Van Blerkom, 2004), reduction in recombination (MacDonald et al., 1994; Hassold et al. 1996) and reduction in levels of cohesin proteins that lead to premature loss of sister-chromatid cohesion (Wolstenholme and Angell, 2000; Garcia-Cruz et al., 2010) in oocytes from older women have all been suggested to lead to increased incidence of aneuploidy.

1.4.2 Structural chromosome abnormalities

Structural chromosome abnormalities occur when fragments of chromosomes break and reunite to form a new configuration. Different types of structural chromosome abnormalities can form this way:

- Translocations involving the breakage and re-joining of chromosome segments. Breakage can affect any parts of two or even more chromosomes. There are two types of translocations, reciprocal and Robertsonian.
    - Reciprocal translocations take place between any two or more chromosomes and involve the exchange of material between them (Figure 1-3).
    - Robertsonian translocations occur when the satellite regions of two acrocentric chromosomes (13, 14, 15, 21 and 22) break, followed by fusion of the long arms. As a result a larger derivative is formed, and the total chromosome number of the carrier is reduced to 45 (Figure 1-4).
- Inversions form due to the breakage and re-insertion of a fragment of chromosome in an inverted manner. The inverted fragment may include the centromere (pericentric inversions) or may not (paracentric inversions).

- Deletions are caused by loss of a chromosome fragment. Deletions of some specific chromosome regions are associated with defined genetic syndromes. Examples include the Wolf-Hirshhorn syndrome caused by the deletion of band p16.3 of chromosome 4, the Cri-du-chat syndrome (deletion of 5p15.33-5p15.2 or the entire p arm of chromosome 5), and the DiGeorge syndrome (small deletion of band q11.2 of chromosome 22).
- Insertions take place when a fragment is lost from one chromosome and inserts itself at another site, usually on a different chromosome.
- Duplications are formed when a part of a chromosome is duplicated, resulting in the presence of extra genetic material.
- Ring chromosomes are formed when both arms of a chromosome suffer breaks, with loss of telomeric regions, leaving behind sticky ends. These tend to reunite forming in this way a ring or circle.
- Isochromosomes involve the loss of one of the two chromosome arms and the duplication of the other (mirror image).

Figure 1-3: A balanced reciprocal translocation is shown. A fragment of the long arm (q) of chromosome A exchanged with a fragment of the long arm (q) of chromosome B

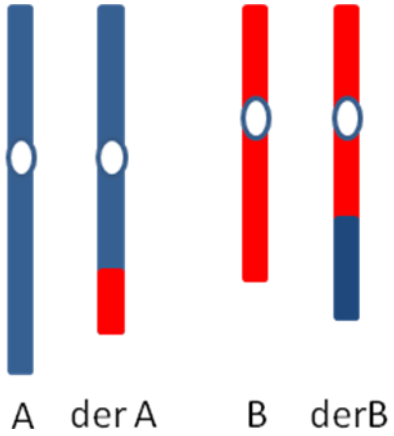
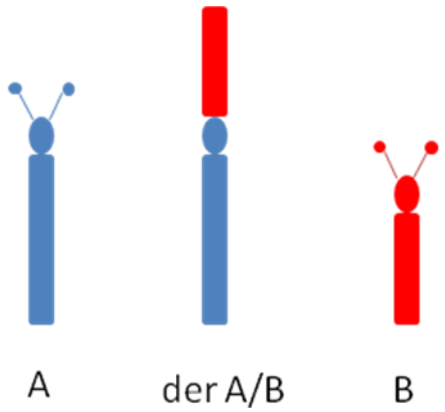


Figure 1-4: A balanced Robertsonian translocation is shown. Acrocentric chromosome A fused with acrocentric chromosome B.



Balanced chromosome rearrangements (CR) represent a relatively common form of genetic abnormality. Translocations (reciprocal and Robertsonian) and inversions are the most frequently occurring types of structural chromosome abnormalities. The frequency of structural abnormalities in prenatal samples and newborns has been determined to be 0.4 and 0.19%, respectively (Jacobs et al., 1974; Van Dyke et al., 1983; reviewed in Simpson and Bischoff, 2002). In most cases, balanced chromosome rearrangement carriers are phenotypically normal, as all the genetic/chromosomal material is present, but the chromosomes involved in the rearrangement are somewhat "modified". Structural abnormalities tend to remain undetected until the carrier attempts to reproduce. At this point the carrier may experience decreased fertility, high rates of miscarriage and in some cases, the birth of children affected by congenital defects and/or mental retardation. These difficulties are due to the problems processing the rearranged chromosomes during meiosis, which frequently produces gametes with missing or extra pieces of chromosome.

For carriers of reciprocal translocations, pairing of homologous fragments of the normal and derivative chromosomes on the meiotic spindle leads to the formation of what is called a (pachytene) quadrivalent (Figure 1-5), instead of the expected bivalent. There are various different ways that this quadrivalent could resolve during meiosis, and these are outlined in Table 1-1.

Figure 1-5: Pachytene quadrivalent formation during meiosis I in carriers of balanced reciprocal translocations

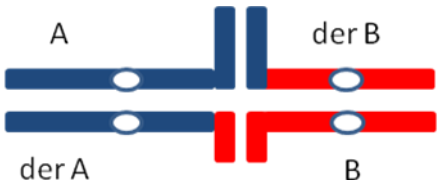


Table 1-1: Different segregation patterns of a reciprocal translocation

Segregation pattern	Carrier chromosomes	Embryo genetic constitution after fertilisation with chromosomally normal gamete	Embryo characterization
Alternate	A and B or	Normal chromosome complement	Normal
	der A and der B	Balanced chromosome complement	Balanced carrier of the translocation
Adjacent-1	A and der B or	Partial trisomy of telomeric A and partial monosomy of telomeric B	Abnormal
	der A and B	Partial trisomy of telomeric B and partial monosomy of telomeric A	Abnormal
Adjacent-2	A and der A	Partial trisomy of centric A and partial monosomy of centric B	Abnormal
	B and der B	Partial trisomy of centric B and partial monosomy of centric A	Abnormal
3:1 interchange	A, der A and der B	Trisomy of A and balanced chromosome complement	Abnormal
	B	Monosomy A	Abnormal
	B, der A and der B	Trisomy of B and balanced chromosome complement	Abnormal
	A	Monosomy B	Abnormal
3:1 tertiary	A, B and der A	Partial trisomy of centric A and partial trisomy of telomeric B	Abnormal
	der B	Partial monosomy of centric A and partial monosomy of telomeric B	Abnormal
	A, B and der B	Partial trisomy of centric B and par-	Abnormal

		tial trisomy of telomeric A	
	der A	Partial monosomy of centric B and partial monosomy of telomeric A	Abnormal
4:0	A, B, der A, der B	Trisomy A and trisomy B and balanced chromosome complement	Abnormal
	0	Monosomy A and monosomy B	Abnormal

A similar type of “altered” meiosis has been described for carriers of Robertsonian translocations. Hence, the two normal chromosomes and the derivative chromosome pair and form the pachytene trivalent (Figure 1-6). However, unlike reciprocal translocations, the resolution of a Robertsonian translocation trivalent is not as complicated. The different segregation patterns are described in Table 1-2.

Figure 1-6: Pachytene trivalent formation during meiosis I in carriers of balanced Robertsonian translocations

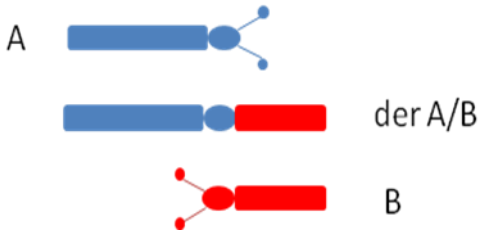


Table 1-2: Different segregation patterns of a Robertsonian translocation

Segregation pattern	Carrier chromosomes	Embryo genetic constitution after fertilisation with chromosomally normal gamete	Embryo characterization
Alternate	A and B	Normal chromosome complement	Normal
	der A/B	Balanced chromosome complement	Balanced carrier of the translocation
Adjacent	A and der A/B	Trisomy A	Abnormal
	B	Monosomy A	Abnormal
Adjacent	B and der A/B	Trisomy B	Abnormal
	A	Monosomy B	Abnormal
3:0	A, B and der A/B	Trisomy A and trisomy B	Abnormal
	0	Monosomy A and monosomy B	Abnormal

As already discussed, even though the incidence of chromosomal rearrangements is appreciable in the general population, they are even more common among infertile patients. Previous studies reported a 25-fold increase (5%) in the frequency of rearrangements carriers in patients attending IVF clinics with a history of recurrent miscarriages or repeated IVF failure (Campana et al., 1986; Frynes and Buggenhout, 1998; Stern et al., 1999). For these infertile patients, the high prevalence of gametes that have lost or gained chromosome material as a result of the rearrangement is likely to be the single most significant factor in their previous pregnancy losses and lack of IVF success (reviewed in Scriven et al., 1998). In general, couples affected by the presence of a chromosome rearrangement, will either attempt to reproduce naturally and opt for

prenatal diagnosis, or regardless of fertility status, could choose to undergo through an IVF cycle, in combination with preimplantation genetic diagnosis.

It has been suggested that, besides the direct effect on the chromosomes involved in the rearrangement, there may also be an increased risk of malsegregation for other, structurally normal, chromosomes during meiosis. This might be due to disruption in the alignment of chromosomes on the meiotic spindle, or interference with other essential aspects of the chromosome segregation process, leading to a generalised increase in the risk of producing chromosomally abnormal gametes. This phenomenon is known as an inter-chromosomal effect (ICE) (Lejeune, 1963). Many researchers have tried to establish whether this phenomenon truly exists, but it has remained controversial. While the existence of an ICE has been described by some studies investigating embryos produced by carriers of chromosome rearrangements undergoing PGD (Gianaroli et al., 2002; Vozdova e t al., 2011), other studies have yielded contrary results, reporting a negligible or entirely absent ICE (Munne et al., 2005). Studies conducted on sperm provide strong data in favour of an ICE for some carriers of translocations, but not others (Anton et al., 2008; 2010). Discrepancies between different studies might be a consequence of variability and limitations of the FISH-based methods used. In all cases, the number of chromosomes assessed was limited. Moreover, Bonnet-Garnier et al. (2009) used the pig as an animal model to examine the presence/absence of ICE. FISH was used for 7 chromosomes (1, 10, 11, 13, 18, X and Y) to analyse sperm samples of 2 boars carrying two different reciprocal translocations and compared the aneuploidy rate to that in sperm samples of fertile boar with a normal karyotype and reported no significant increase in aneuploidy rate in sperm from carriers of translocations which implies no presence of ICE (Bonnet-Garnier et al., 2009). Also, Chinese hamster with reciprocal translocation was studied as an animal model to investigate ICE in meiotic cells and one-cell embryos and failed to find an increased incidence of aneuploidies affecting chromosomes not involved in the rearrenamgent (Sonta and Kitayama, 1991).

1.5 Preimplantation genetic diagnosis (PGD)

Preimplantation genetic diagnosis can be considered an alternative to prenatal diagnosis in cases of couples at high risk of transmitting a genetic disorder or a chromosomal abnormality to their offspring. It involves the genetic analysis of one or more cells biopsied from oocytes or embryos, generated by *in vitro* fertilization (IVF) (Handyside and Delhanty, 1997). PGD was first successfully used for two couples at risk of transmitting an X-linked disorder (adrenoleukodystrophy and X-linked mental retardation) to their offspring (Handyside et al., 1990). The detection of a Y chromosome specific-sequence enabled the transfer of unaffected female fetuses (Handyside et al, 1990).

Couples requesting PGD have to undergo an in vitro fertilization (IVF) cycle so as to generate embryos which will then be genetically tested (Handyside et al., 1990). This genetic testing takes place by obtaining cellular material with the use of biopsy. The biopsy procedure could take place on the oocyte (removal of 1^{st} and 2^{nd} polar bodies) if the carrier of the genetic disorder is the female partner, or on the actual embryo. Embryo biopsy can be undertaken either at the cleavage stage where 1-2 blastomeres are removed, or at the blastocyst stage where a clump of trophectoderm cells are taken. Normal embryos are transferred to the uterus after performing PGD, leading to elimination of the need to terminate an affected pregnancy following prenatal diagnosis.

1.5.1 Biopsy methods

Genetic analysis of preimplantation embryos requires opening of the zona pellucida followed by removal of the embryonic cellular material. The main methods used to achieve this are mechanical, chemical or laser-assisted procedures.

1.5.1.1 Mechanical zona opening procedure

Three main methods have been described and employed for mechanical zona opening. The first method involves the use of a bevelled pipette and requires direct puncture of the zona. This approach has proven very efficient for polar body biopsy (Verlinsky and Cieslak, 1993) but has not been used for embryos at the cleavage or blastocyst stage. The second method is known as partial zona dissection (PZD) and involves creating a slit in the zona using a sharp, closed micro-needle. This method was first used to facilitate sperm penetration through the zona (Cohen et al., 1989) and also to assist embryo hatching (Grifo et al., 1990). The third approach is a new method of PZD and involves the creation of a cross-shaped opening in the zona by making 2 perpendicular slits with a sharp, closed micro-needle. This enables the easy removal of 1st and 2nd polar bodies. In the case of cleavage stage biopsy and blastomere removal, the opening has a V shape (Cieslak et al., 1999). Mechanical zona opening procedures are generally considered safe and simple for both polar body and embryo biopsy (De Vos and Van Steirtegham, 2001).

1.5.1.2 Chemical zona drilling

Chemical drilling of the zona involves the use of acidic Tyrode's solution (pH 2.3). This procedure leads to the creation of a large, round hole in the zona and it is not easy to control the size of the opening. Chemical zona drilling has proven inappropriate for polar body biopsy as it compromises the viability of the oocyte (Payne et al., 1991). On the other hand, this method was successfully used for cleavage stage embryo biopsy (Grifo et al., 1992, 1994). The use of acidic Tyrode's solution may increase the risk of culture medium acidification (Garrisi et al., 1990) and cell lysis and may interfere with further embryo development (De Vos and Van Steirtegham, 2001). Nonetheless, pregnancy and birth rates generally appear to have not been compromised following its application for cleavage stage biopsy (Harper, 1996; Joris et al., 2003; Jones et al.,

2006). The procedure itself is simple but requires technical skills to ensure an appropriate size of the hole while limiting the duration of embryo exposure (De Vos and Van Steirtegham, 2001).

1.5.1.3 Laser assisted zona opening

Laser assisted zona opening has been the most common used biopsy method in recent years (Harper et al., 2012). It involves the use of laser beams; electromagnetic waves with wavelengths ranging from the ultraviolet (UV) to the infrared (IR), to allow zona drilling.

The use of UV lasers may have severe mutagenic effects to the oocyte/embryo. In order to avoid this risk, the desirable laser wavelength is adjusted sufficiently far from the maximum DNA absorption. Thus, laser in the infrared region (1.48 μm diode laser) was evaluated for safety and efficacy (Germond et al., 1995) and used for polar body/embryo biopsy and assisted hatching, without affecting further embryonic development and pregnancy rates (Veiga et al., 1997; Boada et al., 1998; Chang et al., 2004; Montag et al., 2004; Jones et al., 2006). The advantages of this method include the ability to precisely control the size of the hole created in the zona (contrary to the chemical breaching of the zona), combined with a minimal damage. This approach provides a simple, less detrimental and extremely accurate zona opening method, but the laser equipment is quite expensive (De Vos and Van Steirtegham, 2001).

1.5.2 Stage of biopsy

The main developmental stages during which biopsy can be performed are: 1st and 2nd polar body biopsy before and after oocyte fertilisation, blastomere biopsy at the cleavage stage and trophectoderm biopsy at the blastocyst stage.

1.5.2.1 Polar body biopsy

Polar body analysis offers an indirect diagnosis of the genetic and chromosomal constitution of the oocyte (Figure 1-7). The first clinical application of first polar body analysis was performed by Verlinsky et al (1990) for a couple where the female partner was a carrier of alpha-1-antitrypsin deficiency.

Figure 1-7: Biopsy of first polar body from an unfertilized oocyte*



*Image courtesy of *Tracey Griffith*, Oxford Fertility Unit

First and second polar body analysis has been used for thousands of PGD cases for single gene disorder carriers (Verlisnky et al., 1997, 1999), for aneuploidy screening (Munne et al., 2000; Kuliev et al., 2003, 2005; Wells et al., 2002; Pujol et al., 2003a; Fragouli et al., 2006; 2010) and for carriers of structural chromosome abnormalities (Munne et al., 1998a; Pujol et al., 2003b).

The advantage of polar body biopsy is that it is a less invasive approach. Polar bodies are not needed for regular embryo development, and therefore their removal should not adversely affect further embryonic development (Kulliev et al., 2010a). However, it only provides information about meiotic abnormalities of female origin and does not reveal those of paternal or post-zygotic origin.

1.5.2.2 Cleavage stage biopsy

Cleavage stage biopsy is the most commonly used method for PGD (ESHRE consortium, 2012). It involves the removal of 1-2 blastomeres from day-3 embryos and, therefore leads to a significant reduction in cell mass (Figure 1-8). Hardy et al (1990) reported that removing blastomeres from cleavage stage embryos does not adversely affect their further development to the blastocyst stage. However, more recent studies did show that the removal of 2 cells from cleavage stage embryos leads to an obvious reduction of their implantation potential (Van de Velde et al., 2000; Cohen et al., 2007; Goossens et al., 2008).

Figure 1-8: Biopsy of a single blastomere from an embryo at the cleavage stage*



*Image courtesy of *Tracey Griffith*, Oxofr Fertility Unit

Another problem associated with biopsy on day-3 of preimplantation development is the presence of tight junctions between blastomeres (Tesarik, 1989; Dale et al., 1991). These junctions may lead to difficulties in the removal of cells, and it may also cause their lysis. To an extent, this issue has been dealt with by using Ca^{++}/Mg^{++}-free medium. This medium loosens cell-cell adhesion (Santalo et al., 1996; Dumoulin et al., 1998) and allows an easier removal of blastomeres. Dumoulin et al (1998) have evaluated the use of Ca^{++}/Mg^{++}-free medium on further embryo development to the blastocyst stage and showed that its use did not have a detrimental effect.

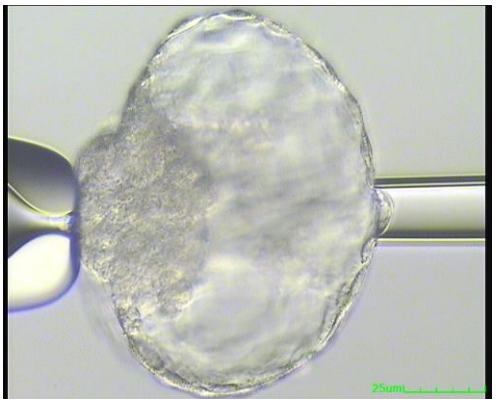
Moreover, chromosome mosaicism is a very common phenomenon in human embryos at the cleavage stage and ranges between 30-85% (Munne et al., 1994; Pellestor et al., 1995; Delhanty et al., 1997; Voullaire et al., 2000; Wells et al., 2000; Vanneste et al., 2009).

1.5.2.3 Blastocyst stage biopsy

Biopsy at the blastocyst stage involves the removal of 3-10 cells from the trophectoderm layer, leaving the inner cell mass intact (Figure 1-9). The higher number of cells available for diagnosis can minimise the risk of error (Veiga et al., 1997). Dokras et al (1990) was the first to investigate the effect of trophectoderm biopsy; performed by mechanical slitting of the zona pellucida opposite the area where the inner cell mass is on day-5 and waiting 18-24 hours for cells to herniate, on further embryo development *in vitro* until day-14 and reported that the biopsy procedure was not detrimental to the embryo. Veiga et al (1997) used 1.48 µm diode laser to create a hole in the zona of day-5 embryos and reported promising recovery rate of the blastocysts. In order to provide the possibility of fresh embryo transfer following genetic analysis of the blastocyst, McArthur et al (2005) reported the application of embryo hatching (creating a hole in the zona) on day-3 or -4 to allow trophectoderm cells to herniate from the blastocyst early on day-5 where they can be biopsied, tested and embryos could be transferred later on day-5 or on day-6. The first live

birth following blastocyst biopsy and PGD analysis was reported in 2002 by De Boer and colleagues (De Boer et al., 2002).

Figure 1-8: Biopsy of trophectoderm cells from an embryo at the blastocyst stage*



The main advantages of day-5 compared to day-3 embryo analysis are firstly, the incidence of chromosome aneuploidy at the blastocyst stage was reported to be lower than that at the cleavage stage (Sandalinas et al., 2001; Fragouli et al., 2008; Santos et al., 2010). Secondly, the implantation rate of embryos biopsied at the blastocyst stage was significantly higher than that achieved following cleavage stage biopsy (all embryos transferred at the blastocyst stage regardless of day of biopsy) (McArthur et al., 2008). Moreover, although the number of embryos that form blastocysts is less than the number that reach the cleavage stage, resulting in a reduction in the number of embryos available for testing and a lower proportion of cycles that have an embryo transfer, the pregnancy rate per oocyte retrieval was higher when biopsy was performed on

day-5 compared to day-3 biopsy (27.7% vs. 19.8%) (McArthur et al., 2008). On the other hand, the incidence of mosaicism, the presence of two or more karyotypically distinct cells line in the same embryo, is higher in cleavage stage (Delhanty et al, 1997; Munné et al, 2002; Katz-Jaffe et al, 2004) compared to blastocyst stage embryos (Evsikove and Verlinsky, 1998; Magli et al, 2000; Bielanska et al, 2002; Santos et al, 2010).

Recent studies have reported an excellent implantation rate following trophectoderm biopsy followed by aneuploidy screening (Schoolcraft et al., 2009; Traversa et al., 2011; Yang et al., 2012), diagnosis for single gene disorders (Kokkali et al., 2007; McArthur et al., 2008) and for carriers of chromosome rearrangements (McArther et al., 2008; Treff et al., 2011).

1.5.3 Application of preimplantation genetic diagnosis

PGD has been applied to detect genetic and chromosomal abnormalities including: detection of single gene disorders, X-linked diseases, chromosome rearrangements and for screening of chromosome aneuploidies.

1.5.3.1 Single gene disorders

PGD has been applied for over 170 single gene disorders in addition to two reported cases for diagnosis of mutations in the mitochondrial genome (ESHRE PGD consortium, 2012). Single gene disorders can be divided into autosomal recessive, autosomal dominant, X-linked disease. The most common indications for PGD of single gene disorders have been for cystic fibrosis (CF), myotonic dystrophy type 1 (DM1), Huntington disease (HD, triplet repeat expansion), β thalassemia and sickle cell anaemia (mutations on the HBB gene), fragile X syndrome (FraX) and spinal muscular atrophy (SMA). In the main, the spectrum of disorders to which PGD has been applied is similar to that of prenatal diagnosis. However, there has been increased utilisation of PGD for the diagnosis of late onset neurodegenerative disorders (such as HD) and inherited cancer predisposi-

tion. Not only has PGD been performed for fully penetrant disorders, but it has also been used for detection of mutations associated with incomplete penetrance (e.g. mutations of the BRCA1 gene) (ESHRE PGD Consortium, 2012).

Another area in which PGD has deviated from traditional prenatal diagnosis is in its application for the purpose of human leukocyte antigen (HLA) typing. Patients with an existing child affected by a lethal hemoglobinopathy [e.g. α- or β-thalassemia, sickle cell anaemia or Diamnond-Blackfan anaemia (DBA)], requiring an HLA-compatible stem cell transplantation, may opt to undergo a cycle of IVF and PGD in order to select unaffected HLA-matched embryos. Upon the birth of the tissue matched child, stem cells can be retrieved from the umbilical cord and transplanted to the affected sibling, in the hope of permanently curing their disorder (Bellavia et al., 2010; Kulliev et al., 2011b; Harper et al., 2012).

Given the minute amount of genetic material available from a single cell, some sort of DNA amplification is essential. In the case of PGD for single gene disorders, this is most commonly achieved using multiplex-PCR to amplify mutation sites and also linked polymorphic markers (Wells and Sherlock, 1998; Gutierrez-Mateo et al., 2009). However, recent years have seen increasing utilization of whole genome amplification (WGA) methods. Techniques for the non-specific amplification of the entire genome are essential for the testing of single cells using microarray-based molecular cytogenetic approaches (discussed below). One of the principal challenges facing PGD methods reliant on DNA amplification is contamination (Wells and Sherlock, 1998; reviewed in Sermon, 2002). With a starting template of only one genome (e.g. one blastomere), the risk of contamination with exogenous DNA is a major problem, necessitating stringent experimental practices. Reagents and equipment used for single cell PCR should never be used for other purposes and amplification reactions must be set-up in a dedicated room, with restricted personnel access and dedicated air flow. Contamination from maternal cumulus cells and sperm are another potential source of contamination, requiring extreme care during post-biopsy washing of

cells. Risks of contamination can be reduced by removing cumulus cells prior to biopsy and performing intracytoplasmic sperm injection (ICSI) for fertilization (Wells and Sherlock, 1998).

1.5.3.2 Sexing for X-linked disease

As mentioned above, the first PGD case was applied to embryos from a couple with an X-linked disorder and the diagnosis was based on the detection of a Y-chromosome-specific fragment using a PCR approach (Handyside et al., 1990). However, this strategy was soon replaced by fluorescent *in situ* hybridisation (FISH), with X and Y chromosome-specific DNA probes (Griffin et al, 1993; 1994). The FISH method has been used much more frequently than PCR for sex selection, as there are several distinct achieved advantages of this approach. The method has been shown to be robust and contamination risk is minimal, as the biopsied cell can be visualized under the microscope before and after the procedure and sperm can be clearly distinguished from nuclei of embryonic origin. Moreover, the use of FISH reveals the copy number of X- and Y-chromosomes and therefore not only indicates the sex of the embryo but also the presence sex chromosome abnormalities (e.g. Tuner and Klinefelter syndromes) (Griffin et al, 1993; 1994; Delhanty et al, 1993).

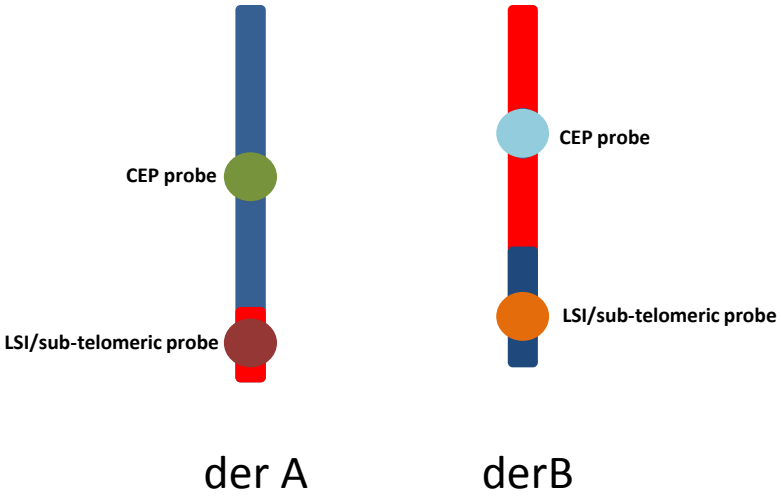
1.5.3.3 PGD for structural chromosome abnormalities

The first report of a pregnancy after PGD for a Robertsonian translocation of maternal origin was performed via first polar body biopsy and analysis using whole chromosome painting probes (Munne et al, 1998b). Since then, more than 4000 cycles of PGD for carriers of balanced chromosome rearrangements have been performed in different centres around the world. More than half of these cases were done for carriers of reciprocal translocations, a quarter for carriers

of Robertsonian translocations and the rest were for other types of chromosome rearrangements (ESHRE PGD Consortium, 2012).

Most chromosome rearrangements are unique, as breakpoints can potentially occur at any point on any chromosome. Robertsonian translocations are an exception, as they are formed only among acrocentric chromosomes. Commercially available chromosome enumeration probes can be used to diagnose polar bodies or embryos generated from carriers of these rearrangements via FISH (Conn et al., 1998). In the case of carriers of reciprocal translocations or inversions, a patient-specific protocol has to be developed where DNA probes on both sides of the breakpoints on the rearranged chromosome(s) (Figure 1-10) (centromeric and locus specific (LSI)/sub-telomeric) are applied first on cultured lymphocytes including interphases and metaphases prepared from the carriers can be used to validate and test the efficiency of the protocol (Van Assche et al., 1999; Pierce et al., 1998).

Figure 1-9: Illustration about probes that need to be used for detection of structural chromosome errors in samples from carriers of chromosome rearrangements



CEP: centromeric. LSI: locus-specific.

The development of these personalized FISH protocols for each type of chromosome re-arrangement is labour intensive and expensive as it is important to make sure that no parental polymorphisms might reduce or eliminate probe hybridization. The other main limitation of using FISH is that it is unable to provide comprehensive chromosome screening.

1.6 Embryo selection

Assisted reproductive technology (ART) includes methods used to assist infertile couples to achieve a pregnancy followed by a healthy live birth. To maximize the success rates of such treatments, a reliable means of recognizing the embryo with the greatest potential to become a healthy child is required. The need to identify viable embryos becomes increasingly important as clinics expand the number of cycles involving single embryo transfer, a measure undertaken in order to reduce the risk of multiple pregnancy. Methods used to select the 'best' embryo can be both non-invasive and invasive. Non-invasive approaches include morphological assessment, metabolomic/proteomic profiling of the follicular fluid and embryo culture medium, and gene expression studies of the cumulus cells. Of these approaches, only morphological analyses are routinely applied in IVF clinics, the others forms of non-invasive testing are considered experimental. Invasive strategies focus on examining the genetics of the embryo and require the biopsy of the oocyte or embryo followed by chromosome screening using any of a variety of techniques (discussed below).

1.6.1 Morphological assessment

Currently, the most commonly used method of embryo evaluation in IVF clinics relies on the assessment of three developmental stages and groups of morphological criteria within these stages. These stages are the zygote just after fertilization, the cleavage on day-3 of preimplantation development and the blastocyst stage on days-5 or -6.

Morphological criteria examined at the zygote stage include assessment of the pronuclear alignment (Van Blerkom et al., 1995), the timing of the second polar body extrusion along with that of male and female pronuclear formation (Payne et al., 1997), the orientation of the polar bodies in relation to the axis of the pronuclei (Garello et al., 1999), and the morphology of the pronuclei (Tesarik and Greko, 1999; Gianaroli et al., 2007).

The morphology of cleavage stage embryos has been most thoroughly investigated in terms of predicting clinical outcomes and is the stage most commonly assessed in IVF clinics. Some morphological features are correlated not only with ability to develop to the blastocyst stage but also with implantation and pregnancy potential. The main characteristics evaluated at the cleavage stage of preimplantation development include the evenness of mitotic divisions (blastomere size), the extent of cellular fragmentation present, the presence of multinucleation and the rate of embryo growth (Cummins et al., 1986; Puissant et al., 1987; Van Royen et al., 1999, 2003; Hardarson et al., 2001; Luna et al., 2008). A day-3 embryo scoring system relying on the number of cells, percentage of fragmentation and multinucleation was proposed by Scott et al., (2007) in order to select the embryo with highest potential (Table 1-3). Most IVF clinics use systems similar to this.

Table 1-3: Cleavage stage embryo scoring system

Embryo grade	Embryo characteristics
1	6-8 blastomeres of equal size and no visible fragmentation or multinucleation
2	6-8 blastomeres of even or uneven shape with less than 10–20% fragmentation, or multinucleation
3	Less than 6 cells of equal size and no fragmentation or multinucleation
4	More than 20% fragmentation or very uneven, incorrect cell size
5	Cleavage arrest or morphologically abnormal embryos

The most widely used scoring system for blastocyst stage embryos was that proposed by Gardner and Schoolcraft (1999), involving the assessment of three main characteristics; the expansion of the embryo, the quality of the inner cell mass and the quality of the trophectoderm (Table 1-4). Further studies from the same group reported a correlation between blastocyst score and elevated implantation and pregnancy rates (Gardner et al., 2000).

Table 1-4: Blastocyst stage embryo scoring system

Blastocyst expansion grade	Interpretation	ICM grade	Interpretation	TE grade	Interpretation
6	Hatched blastocyst- the blastocyst has completely escaped the zona pellucida	A	Tightly packed with many cells	A	Many cells forming a cohesive epithelium
5	Hatching blastocyst- trophectoderm (TE) cells have begun to herniate through the zona pellucida	B	Loosely grouped with several cells	B	Few cells forming a loose epithelium
4	Expanded blastocyst-the blastocoel is now larger than the early embryo, and the zona pellucida has begun to thin	C	Very few cells	C	Very few large cells.
3	Full blastocyst- the blastocoel fills the embryo completely				
2	Blastocyst- the blastocoel occupies more than one-half of the volume of the embryo				
1	Early blastocyst-the blastocoel accounts for less than one-half of the volume of the embryo				

There are however some problems associated with the use of the above mentioned morphological criteria as the sole guide to embryo quality. Studies have found that almost 50% of cleavage stage embryos with good quality are chromosomally abnormal (Magli et al., 2007). Therefore, it is possible that a 'good quality' embryo will be genetically incapable of implantation

or to production of a live birth. Additionally, embryos of poor morphological quality can sometimes succeed in producing a healthy child. It is universally acknowledged within the field of IVF that, despite thirty years of research and refinement, morphological scores remain only weakly associated with pregnancy rates.

Very recently, the use of time-lapse monitoring, using cameras incorporated inside incubators, has not only allowed the reduction of stress on the embryo produced from out-of-incubator observation (Zhang et al., 2010), but also provided a more dynamic evaluation of pre-implantation development parameters (Kirkegaard et al., 2012). Wong et al. (2010) reported the possibility of predicting blastocyst formation potential of embryos using parameters assessed up until day-2 of embryonic development (before embryonic genome activation takes place). In their study, they showed that embryos that develop to the blastocyst stage follow a predictable development timeline which enabled the researchers to create an algorithm which includes different imaging parameters to predict blastocyst formation by the 4-cell stage. This system is now known as early embryo viability assesement (EEVA). A recent study has shown that the use of EEVA systems in combination with cleavage-stage morphology assessment significantly improved their ability to select embryos that will form blastocysts of good quality (Conaghan et al., 2013).

Moreover, a retrospective analysis of time-lapse images of 247 transferred embryos showed a correlation between timing of divisions, multinucleation and uneven blastomere size and implantation potential (Meseguer et al., 2011). However, a recent randomized control trial, comparing IVF implantation and pregnancy rates for embryos cultured in a conventional incubator with those cultured in a time-lapse incubator system, reported no significant differences in implantation or pregnancy rates (Kirkegaard et al., 2012). It is possible that time-lapse analysis of embryos will add extra subtlety to morphological analysis, permitting enhanced embryo selection, but current data suggests that it is still a long way from allowing an absolute determination of an embryos ability to implant.

Furthermore, rapid imaging of freshly fertilized mouse oocytes in combination with image analysis based on particle velocimetry showed that fertilization induces repetitive cytoplasmic movements caused by contractions of the actomyosin cytoskeleton correlated with Ca^{2+} oscillations (Ajduk et al., 2011). Analysis of this cytoplasmic flow showed correlation with the developmental potential of embryo. Embryos exhibiting very frequent cytoplasmic movements and low cytoplasmic speeds in intervening periods were significantly less successful in developing to live births compared to those that showed average values (Ajduk et al., 2011).

1.6.2 Preimplantation genetic screening (PGS)

The assessment of chromosome copy number in embryos produced by karyotypically normal patients, with the aim of preventing the transfer of those that are aneuploid and increasing the chance of a healthy pregnancy, is known as preimplantation genetic screening (PGS) (Geraedts et al., 2001). The idea of screening for aneuploidy in order to improve IVF success rates is based upon the fact that chromosome abnormalities have a major role in human reproductive failure. Around 50% of pregnancies that miscarry before 13 weeks (Hassold and Jacobs, 1984; Menasha et al., 2005) and approximately 0.3% of live births are chromosomally abnormal (Hassold and Jacobs, 1984). Moreover, it has been estimated that the risk of aneuploidy at conception is about 12%, but it increases to more than 50% for women over 40 years (Hassold and Jacobs, 1984). The risk of miscarriage is also closely associated with maternal age. A retrospective study of spontaneous abortions carried out over a period of 12 years reported an increase in the aneuploidy rate from 36% for maternal age <25 years to 78% for maternal age of ≥40 years (Menasha et al., 2005).

Following the successful application of FISH for sex determination (Griffin et al., 1992, 1993, 1994), protocols were introduced that allowed an increased number of chromosomes to be tested. Initially, embryos from couples undergoing infertility treatment were analysed using FISH to assess five chromosomes frequently implicated in miscarriage and chromosomal syndromes (13, 18, 21, X and Y) (Munne et al., 1993). Women of advanced reproductive age were one of the first groups of patient to be offered such screening (Munne et al., 1993; Delhanty and Harper, 2000). The hope was to reduce the rates of miscarriage and chromosomal syndromes (principally Down's syndrome) and increase implantation and pregnancy rates.

Since the first attempts to use PGS clinically, numerous studies using FISH have confirmed the high incidence of chromosome abnormalities in human oocytes (e.g. Munne et al., 1995a, 2000; Verlinsky et al., 1995, 1996, 1997; Mahmood et al., 2000), embryos at the cleavage stage (e.g. Munne et al., 1995b; Delhanty et al., 1997; Gianaroli et al., 1997) and blastocysts (Evsikov and Verlinsky, 1998; Ruanvutilert et al., 2000; Coonen et al., 2004; Daphnis et al., 2005). Additionally, the indications for PGS have been extended to include other groups as well as those of advanced reproductive age. Currently, the main indications for PGS are as follows (Harper et al, 2006):

- Advanced female age (the threshold used varies, but is generally between 35 and 40 years)
- Repeated IVF failure (RIF) (three or more failed IVF attempts).
- Repeated miscarriages (RM) (two- or more spontaneous abortions).
- Severe male factor (SMF) infertility (abnormal semen parameters).

Chromosome screening is attractive as a diagnostic for the identification of embryos of high developmental competence, as aneuploidy is almost always lethal and is therefore likely to have a more definitive impact on viability than either morphologic or metabolic features. The main methods that have been applied to screen human oocytes and embryos for aneuploidy are

fluorescent *in situ* hybridization (Verlinsky et al., 1998, 2003, 2004; Munne et al., 1993, 2010; Harper et al., 1995; Gianaroli et al, 1997, 1999; Kahraman et al. 2000; Colls et al., 2009), comparative genomic hybridization (metaphase and microarray)(Wilton et al., 2001; Wells et al., 2002; Fragouli et al., 2010; Schoolcraft et al., 2010; Geraedts et al., 2011; Gutierrez-Mateo et al., 2011) and more recently real-time PCR (Treff et al., 2012; Forman et al., 2012).

1.6.2.1 Fluorescent *in situ* hybridization (FISH)

FISH is a molecular cytogenetic method which uses fluorescently labelled DNA probes complimentary to specific chromosomal regions. It allows the enumeration of chromosomes in interphase and metaphase nuclei. FISH was first applied successfully by Griffin et al (1992) to determine the sex of cleavage stage embryos by using labelled probes for chromosomes X and Y, simultaneously. Ever since that initial application, thousands of human gametes (oocytes and sperm) (Verlinsky et al., 1999, 2003, 2004; Munne et al., 2000; Pujol et al., 2003a; Griffin et al., 1995, 1996; Rives et al., 1998; Kearns et al., 2000; Tempest et al., 2004) as well as cleavage and blastocyst stage embryos have had their chromosome complement analysed using FISH (Munne et al., 1993; Harper et al., 1995; Gianaroli et al., 1997; Colls et al., 2009; Munne et al., 2010; Evsikove and Verlinsky, 1998; Magli et al., 2000; Coonen et al., 2004, Daphnis et al., 2005, 2008; Bielanska et al., 2005; ESHRE PGD consortium, 2012).

During the first few years, PGS for the selection of euploid embryos was most often carried out at the cleavage stage of preimplantation development. A single blastomere would be biopsied, fixed on a microscope slide and examined with the use of up to 3 rounds of FISH. A total of 5-12 chromosomes were targeted, their selection based upon frequencies of aneuploidy observed during analysis of products of conception and prenatal samples. Hence, the most common FISH-PGS protocols usually examined chromosomes 13, 15, 16, 18, 21, 22, X and Y, and on some occasions others such as 14, 17, 19 and 20 (Colls et al., 2009; Munne e t al., 2010). Results obtained from early PGS investigations using FISH, were suggestive of an increase in implantation

and live birth rates (Munne et al., 1999, 2003; Gianaroli et al, 1997, 1999; Kahraman et al. 2000) as well as a reduction in miscarriage rates (Munne et al., 2005; Schoolcraft et al., 2008). However, the studies were criticised for a lack of randomisation and control groups that were sometimes considered inappropriate. More recently, a large number of randomised controlled trials (RCTs) (around 10 trials) reported that PGS at the cleavage stage using FISH does not improve IVF outcome and could possibly reduce the implantation rate of screened embryos (Staessen et al., 2004; Maestenbroke et al., 2007; Blockeel et al., 2008; Hardarson et al., 2008; Meyer et al., 2009; Debrock et al., 2010).

The main drawback of applying FISH as a screening method for PGS is the limited number of chromosomes that can be assessed (up to 12 chromosomes). This is mainly due to the limited number of fluorochromes that can be used to label these DNA probes. Additionally, the sequential rounds of analysis result in a gradual degradation in the integrity of the fixed nucleus, increasing the risk of errors due to probe hybridisation failure (Liu et al., 1998). Moreover, the spreading of embryonic nuclei on microscope slides requires experience and skill, and overlapping or split signals as a result of spreading might lead to misdiagnosis (reviewed in Wilton, 2005).

1.6.2.2 Metaphase comparative genomic hybridization (metaphase CGH)

CGH is molecular cytogenetic technique which allows the simultaneous analysis of the entire chromosome complement. This method was developed and initially applied by Kallioniemi et al (1992) to detect chromosome copy number changes in solid tumours. It involves labelling of a sample 'test' and a chromosomally normal control 'reference' DNA (from a chromosomally normal individual) with two different fluorochromes (by convention- green for the sample being tested and red for the reference) followed by their co-hybridisation to chromosomally normal metaphase chromosomes. A ratio of green:red of 1:1 refers to an equal amount of chromosomal material between the sample and reference DNA. A shift in the ratio to ≤0.8 refers to a loss of

chromosomal material in the sample and a shift to ≥ 1.2 refers to a gain of chromosomal material in the sample.

The first application of CGH for the analysis of single cells was reported by Wells et al (1999). Single cells contain around 6-10 pico grams of DNA whereas CGH requires the labelling of at least 0.2-1 μg of DNA. Thus, whole genome amplification is a pre-requisite step in order for CGH to be applied successfully. The degenerate oligonucleotide primed polymerase chain reaction (DOP-PCR) was the method that led to the best results of WGA followed by CGH. Application of this technique on single blastomeres was reported by Wells and Delhanty (2000) and Voullaire et al (2000). These studies determined for the first time the true extent and type of chromosome abnormalities at the cleavage stage, suggesting that approximately three-quarters of embryos are aneuploid. The first clinical application of CGH for PGS purposes took place in 2001 and was carried out by Wilton and colleagues. A total of 5 embryos generated by a couple with repeated implantation failure were biopsied at the cleavage stage. A normal embryo was identified after CGH analysis, subsequently transferred, and resulted in the birth of a healthy child (Wilton et al., 2001). As the hybridization time required for metaphase CGH to yield results was longer than the embryos could be maintained in culture, all of them were cryopreserved and those found to be euploid thawed and transferred in a subsequent cycle.

Metaphase CGH was first applied to the clinical screening of polar bodies in 2002 by Wells and colleagues. During this investigation ten first PBs were biopsied from oocytes coming from an older (41 years) poor prognosis patient, and a modified rapid CGH protocol, capable of yielding results in approximately 25-30 hours was applied. One of these 10 polar bodies was identified as normal, the corresponding embryo was transferred but no pregnancy was followed. The application of this rapid metaphase CGH protocol and the analysis of polar bodies circumvented the need for embryo freezing. However, only maternally derived meiotic errors from the first meiotic division could be assessed. Several other studies have validated and clinically applied metaphase CGH for the analysis of polar bodies (Gutierrez-Mateo et al., 2004; Fragouli et al., 2006a, b; 2011).

More recently, metaphase CGH was applied to assess trophectoderm cells biopsied from blastocysts (Fragouli et al., 2008). The aneuploidy rate reported at the blastocyst stage was lower than that at the cleavage stage, although still significant (39% for an average female age of 36.5 years) and aneuploidy was seen to potentially affect any chromosome. Similar to the cleavage stage, blastocyst chromosome screening using metaphase CGH required embryo freezing to provide enough time for the analysis to take place.

Even though metaphase CGH proved a very useful method and enabled the first comprehensive cytogenetic analysis of oocytes and embryos, it has various disadvantages. Specifically, metaphase CGH is relatively labour intensive, requires a lot of experience and the hybridization time is very long (48-72 hours). Moreover, metaphase CGH cannot detect ploidy errors (i.e. haploidy, triploidy, tetraploidy, etc.) (Wilton, 2005).

1.6.2.3 Microarrays

Similar to metaphase CGH, most microarray-based methods involve the competitive hybridisation of differentially labelled test and reference DNA samples to DNA probes affixed to a solid support such as a microscope slide (instead of metaphase spreads). Each of these DNA probes is specific to a different region along the length of all 23 chromosomes, and occupies an individual spot on the slide. One of the main advantages that microarrays have over metaphase CGH is shorter hybridisation times and a degree of automation as regards analysis. Together, these features make possible comprehensive chromosome analysis within a 24 period, an important benefit in terms of utilisation for PGD and PGS.

1.6.2.3.1 Microarray CGH platform using Bacterial artificial probes

The most widely used microarray-CGH (aCGH) platform for PGD/PGS employs glass slides upon which thousands of discrete spots composed of DNA derived from bacterial artificial chromosomes (BACs) have been deposited. Each of the BAC clones covers a relatively large chromo-

somal area, typically in the region of 150–200 kb. The first clinical application of a BAC aCGH platform for the purposes of PGD was reported by Fishel et al (2010), using the 24Sure microarray produced by BlueGnome (Cambridge, UK). In a pilot study, comprehensive chromosome analysis was applied to 1st polar bodies biopsied from the oocytes of a 41 year old female who had experienced 13 failed IVF cycles. A singleton pregnancy was achieved following the transfer of two embryos whose corresponding 1st PB were chromosomally normal and a healthy baby was born. During the course of this project, a proof of principle study using the BlueGnome microarray platform was undertaken in two laboratories, organised by the European Society of Human Reproduction and Embryology (ESHRE). This study involved the analysis of 1st and 2nd polar bodies plus their corresponding oocyte if one the PBs was found to be chromosomally abnormal. The data produced indicated that the chromosomal status of the zygote can be predicted using aCGH with an acceptable level of accuracy (~94%) (Geraedts et al., 2011; Magli et al., 2011). Moreover, this aCGH platform was validated on single blastomeres biopsied from day-3 embryos, with results subsequently confirmed by re-analysing aneuploid embryos by subjecting multiple cells to FISH. Using this strategy the error rate for diagnosis at the cleavage stage was shown to be only 2% (Gutierrez-Mateo et al., 2011). A recent randomised pilot study, compared implantation and pregnancy rate of traditional blastocyst transfer cycles (selection of embryos based upon morphology only) with cycles in which euploid embryos had been chosen for transfer following screening using aCGH (Yang et al., 2012). Encouragingly significantly higher implantation and pregnancy rates were reported.

1.6.2.3.2 Microarray CGH using synthetic oligonucleotide probes

Another class of microarrays used for CGH utilise oligonucleotides that are synthesized directly on the surface of the solid support that forms the base for the microarray. Probe lengths range between 25-85 nucleotides depending on the manufacturer. The main advantages of these

arrays are the high reproducibility of the probes as a result of the manufacturing process, as well as the ability to create extremely high-density arrays.

Oligonucleotide arrays have been successfully employed for the detection of chromosome abnormalities in single cells isolated from chromosomally abnormal cell lines and blastomeres from cleavage stage embryos (Le Caignec et al., 2006). Moreover, the first ever application of microarray-CGH, reported by Hellani et al (2008) involved the use of an oligonucleotide microarray manufactured by Agilent and resulted in the first pregnancies achieved after screening with a microarray.

While oligonucleotide-based microarrays share many of the advantages of BAC arrays, hybridisation times are generally longer (and therefore less convenient for PGD). Additionally, the small size of individual oligonucleotide probes increases the risk that artifactual losses and gains will be detected, a consequence of DNA amplification biases introduced during the WGA.

1.6.2.3.3 Single nucleotide polymorphism microarrays

Single nucleotide polymorphisms (SNPs) are common genetic variations, involving substitution of one nucleotide for another, and are found throughout the genome. SNP microarrays are a type of oligonucleotide array that can be used for interrogating individual SNPs, revealing the genotype of the DNA sample under analysis. Most SNP microarray platforms analyse between 10,000 and 500,000 individual SNPs.

Unlike the platforms previously described, where differentially labelled sample and reference DNAs are hybridized simultaneously onto the array, in the case of SNP arrays, the sample DNA is usually hybridized on its own and then compared to a reference DNA library, created from individuals with known normal karyotype and analysed on the same platform. The testing of parental DNA along with samples from embryos provides a way of determining which parental chromosomes were inherited by the embryo. Monosomies are revealed, not only by reduced av-

erage fluorescence seen for all of the probes mapping to the affected chromosome, but also by the presence of only one allele for all SNPs along the affected chromosome (homozygosity, or more accurately- hemizygosity). Conversely, trisomies are revealed by increased mean-fluorescence for probes on the affected chromosome and the detection of three distinct parental haplotypes (i.e. blocks of linked polymorphisms from individual chromosomes that are inherited together). However, unlike microarray CGH, SNP microarrays may have some issues with the detection of trisomies originating in meiosis II. In the absence of meiotic recombination, non-disjunction taking place during the second meiotic division can lead to two identical chromatids entering one of the gametes. Hence, the expected differences in the haplotype will not be present. In such instances, detection of trisomy is entirely reliant upon careful measurement of fluorescence intensity of probes for the affected chromosome.

A significant advantage of SNP-based microarray platforms is that the analysis of SNPs not only provides chromosome copy number information, but also genotyping data. The analysis of multiple polymorphisms produces a DNA fingerprint for each embryo tested, helping to avoid transfer of embryos to the wrong parents and revealing if DNA contamination has occurred during the amplification procedure. Moreover, in cases where more than one embryo is transferred, yet only one gestation ensues, it is possible to determine which embryo implanted and formed a pregnancy.

SNP-based microarrays have been validated on blastomeres from human embryos by Treff et al (2007) and applied clinically on polar bodies derived from oocytes of carriers of chromosome rearrangement (Scott et al., 2012), blastomeres from cleavage stage embryos (Vanneste et al., 2009) and trophectoderm cells biopsied from embryos at the blastocyst stage (Schoolcraft et al., 2011).

Another advantage of SNP-based microarray platforms is the ability of SNP analysis to indirectly detect the inheritance of disease causing mutations through genotyping SNPs located in close proximity to the gene (i.e. linkage analysis). Handyside et al (2008), in their proof of princi-

ple study, demonstrated the ability of a SNP-based microarray to detect the inheritance of mutation-associated haplotypes in single cells, an approach that they have termed 'Karyomapping'. Even though allele dropout rates per locus are relatively high after WGA, an accurate diagnosis can still be obtained because such a large number of informative linked SNPs are tested (Handyside et al., 2008; Rabinowitz et al., 2008).Thus, SNP-based microarrays have the potential to overcome a long standing challenge for single cell analysis- the simultaneous diagnosis of single gene mutations and aneuploidy from the same biopsied sample.

The principal limitations of current SNP-based microarrays are that they are somewhat more expensive than BAC array platforms used for CGH and that the method takes longer to perform, making it difficult to fit within the 24 hour period preferred for PGD tests. However, future developments may help to address both these issues.

1.6.2.4 Quantitative real-time polymerase chain reaction assay

Quantitative real-time PCR was first described by Higuchi et al (1993) were they described the use of a video camera that detects the accumulation of double stranded DNA in each PCR using increase in the fluorescence of ethidium bromide as a result of its binding to the double stranded DNA. This method provides sensitive and quantitative detection of DNA sequences in a dynamic manner. The copy number for all chromosomes can be assigned using standard methods of relative quantification with results for normal male sample used as the reference (Forman et al., 2012).

Treff et al (2012) described the validation of qPCR in a group of cells (5 cells) from cell lines with known karyotypes and in non-transferred embryos previously diagnosed by SNP-based microarrays as abnormal. The same group reported the use of qPCR for selection of embryos at the blastocyst stage along with single embryo transfer and observed an increase in the implantation rate and decrease in the miscarriage rate compared to embryo transfer after morphological assessment only (Forman et al., 2012).

The main advantage of this method is the rapid turnaround time for results to be ready (~4 hours). This is particularly valuable if testing of blastocysts followed by a fresh embryo transfer is desired, since the window of time available for diagnostics to be performed is extremely narrow (Treff et al., 2012; Forman et al., 2012). The main drawback of the qPCR approach is low scalability, meaning that in order to simultaneously analyse several embryos (as is typically needed for a PGD case), multiple real-time thermal cyclers are needed.

1.7 Aims of the project

- This study set out to perform an extensive investigation of the genesis and types of aneuploidy seen during early preimplantation development. A variety of comprehensive cytogenetic methods was employed for this purpose. One of the main purposes of this work was to fill in an obvious gap in knowledge of preimplantation cytogenetics, and test the hypothesis that the frequency of aneuploidy decreases as embryos develop to the blastocyst stage along with mosaicism rate. Prior to this investigation, chromosomal data from human blastocysts using comprehensive methods was extremely scarce. This stage of development is particularly interesting given that it is the time when the first cellular differentiation has just occurred and the embryo is preparing to implant. Three different cytogenetic methods, namely FISH, CGH and aCGH were applied during this part of the project. The use of multiple methods on the same samples also provided an opportunity to perform essential validation of the new aCGH method on material from blastocysts.
- The second aim of the project was to to test the hypothesis that aneuploidy has any adverse effects on embryo morphology at two different stages of preimplantation development, the cleavage and blastocyst stage. Both metaphase CGH and aCGH were used during this part of the research in an attempt to establish a correlation between morphology and aneuploidy. Any potential association would be of biological interest and might have clinical value in terms of screening of embryos for chromosomal anomalies.
- The third aim of the project was to evaluate a universally applicable method for the PGD of chromosome rearrangements (translocations and inversions), as an alternative to patient-specific diagnostics based upon FISH. The microarray-based methodology permitted PGS (i.e. generalised chromosome screening) in addition to detection of imbalances related to individual chromosome rearrangements. Moreover, the hypothesis that chromosome rearrangements influence the segregation of structurally normal chromosomes, or what is known as interchromosomal effect (ICE), was investigated.

- The fourth aim of the project was to test the hypothesis that different infertile patient group benefit from PGS using CGH and aCGH at different stages of development.

Results of the investigations are presented in chapters (3, 4, 5 and 6). In chapter 3, mosaicism at the blastocyst stage was investigated along with the evaluation of aCGH in detecting chromosome abnormalities at the latest stages of embryonic development *in vitro*. Chapter 4 describes the correlation between aneuploidy and embryo morphology at the cleavage and blastocyst stages. Chapter 5 involves the application of metaphase CGH and aCGH for the detection of unbalanced chromosome rearrangements in embryos from carriers of translocations and inversions along with aneuploidies affecting other chromosomes and considers the possible existence of an interchromosomal effect. Chapter 6 focuses on the clinical outcomes and incidence of aneuploidy in embryos from infertile patients. A detailed discussion of the findings of this project along with a review of similar studies and a general conclusion are presented in chapter 7.

Work presented in this thesis has led to the publication of several papers and presentations at international meetings. These are listed in Appendix E.

2. Materials and Metods used during this project

2.1 Ethical approval

Participating IVF centres located in the UK had the appropriate ethical approvals and where necessary a Human Fertilization and Embryology Authority (HFEA) research licence. Local ethics committee approval was also obtained (10/H0606/7). Signed, informed, consent was gathered for all patients contributing samples by the clinics involved. Additionally, this work was approved by the Institutional Review Boards of the IVF centres based in the USA and informed consent was a pre-requisite before donation of non-transferred blastocysts. For retrospective analysis of clinical data (e.g. associating aneuploidy and patient age/embryo morphology) it was determined that no additional ethical approvals were necessary.

2.2 Embryo culture, biopsy and preparation of cells for different tests

Standard methods of controlled ovarian stimulation, IVF/ICSI procedures and embryo culture were used by different participating IVF clinics.

A laser (Hamilton Thorne) was used to create a small opening in the zona pellucida (~ 5 µm) of the examined embryos and 1 blastomere was removed/aspirated from day 3 embryos (cleavage stage) or 3-10 TE cells from day 5 embryos (blastocyst stage). Experienced and certified embryologists from different IVF clinics performed the biopsy.

Biopsied cells were washed by the same embryologist in sterile phosphate-buffered saline (PBS) solution/0.1% polyvinyl alcohol (PVA), placed in 0.2 ml microcentrifuge tubes and sent immediately to our laboratory to be tested or stored at -80˚C until processing.

2.3 Metaphase Comparative genomic hybridization (CGH)

Metaphase CGH is a molecular cytogenetic technique capable of examining the entire chromosome complement of a DNA sample of unknown karyotype (test), by comparing it against

a chromosomally normal reference DNA sample (46,XY or 46,XX). CGH generally requires at least 200ng of DNA whereas a single cell contains 5-10pg of DNA. Therefore, an initial whole genome amplification (WGA) of the test and reference DNA samples is necessary prior to their labelling with two different fluorochromes (green for the test DNA sample, red for the reference DNA sample) and their co-hybridisation on metaphase target slides. WGA took place using the degenerate oligoneclutiode primed-PCR (DOP-PCR) method and fluorescence labelling occurred using a nick translation approach (Nick Translation Kit, Abbott). Once hybridisation was complete, slides were observed under Olympus BX61 fluorescence microscope with a cooled charge-coupled device (CCD) system. CGH analysis was carried out with the use of a specialised computer software package (CytoVysion, 3.9 Applied Imaging).

2.3.1 Degenerate oligoneclutiode primed-PCR (DOP-PCR)

DOP-PCR is a WGA method utilizes degenerate primers containing specified sequences at the 5´ and 3´ ends and a random hexamer between them (CCGACTCGAGNNNNNNATGTGG). Two different phases of primer annealing and amplification are employed; a low-temperature annealing and extension step (8 cycles) followed by a high-temperature step (40 cycles). In the initial phase (low stringency), the low temperature (30˚C) allows binding of the primer to many sites throughout the genome that are complementary to the defined sequence at the 3´ end. The random six nucleotide sequence adjacent to the specified 3´ end helps in stabilizing its annealing. In the following step (high stringency), the elevated annealing temperature (56˚C) increases priming specificity, providing an exponential amplification of the fragments amplified during the earlier low stringency cycles (Telenius et al, 1992; Wells et al, 1999).

Test DNA samples were lysed by adding 2 µl of Proteinase K (125 µg/ml, Roche, USA) and 1 µl sodium dodecyl sulfate (17 mM, Sigma Aldrich, USA) [Appendix A] followed by incubation at 37°C for 1 hour and 15 minutes at 95°C.

Amplifications for test as well as reference DNA samples took place in 50µl reactions containing 0.2 mM dNTPs (HT healthcare,), 2 µM DOP primer (CCGACTCGAGNNNNNNATGTGG) (Invitrogen), 1x SuperTaq Plus buffer and 2.5 U SuperTaq Plus (HT healthcare). Tubes were incubated in an Eppendorf AG 22331 thermal cycler (Germany) and the programme shown in Table 2-1 was followed. Reference DNA was extracted from lymphocytes of karyotypically normal males or females and then diluted to match the DNA content of ~100 cells (1ng).

Table 2-1: DOP-PCR amplification

Temperature	**Time**	**Number of cycles**
94˚C	3 minutes	1 cycle
95˚C	30 seconds	8 cycles
30˚C	1 minute	
68˚C	3 minutes	
94˚C	30 seconds	40 cycles
56˚C	1 minute	
68˚C	3 minutes	
4˚C	Hold	1

After the amplification was complete, precipitation of amplified DNA (45 µl of the product) took place so as to remove unused reagents, purifying the PCR products. DNA precipitation occurred by adding 4.5 µl 3M Sodium Acetate (Sigma Aldrich) and 125 µl absolute Ethanol (molecular biology grade) (Sigma Aldrich) into each of the amplified products. These were then placed in a freezer at -80˚C overnight, and the following day were centrifuged at 13,000 rpm for 30

minutes. The resulting supernatant was removed and the pellet air-dried and re-suspended in 17.5 µl nuclease-free water.

2.3.2 Fluorescence labelling of amplified products

Amplified test and reference DNA samples were subjected to fluorescence labelling via nick translation (Vysis/Abbott). This procedure incorporates differentially labelled nucleotides in the test (spectrum green, SG, dUTP) and reference samples (spectrum red, SR, dUTP).The labelling reaction took place in a total volume of 50 µl including 2.5 µl of 0.2mM fluorescently labelled dUTP, 5 µl of 0.1mM dTTP, 10 µl of a nucleotide mixture (composed of 0.1mM dATP, dGTP and dCTP), 5 µl 10x Nick translation buffer and 10 µl Nick translation enzyme (Vysis/Abbott). All tubes were incubated for 75 minutes at 15˚C and the nick translation reaction was then stopped by increasing the temperature to 70˚C for 10 minutes. This incubation took place in an Eppendorf AG 22331 thermal cycler (Germany).

Test and reference DNAs were then mixed together (50µl each), along with 30µl Human Cot-1 DNA (Invitrogen) and precipitated with the addition of 13µl sodium acetate (3M) and 360µl ethanol (absolute for molecular biology, Sigma Aldrich). The samples were placed in a freezer at -80˚C for one hour and then centrifuged at 13,000 rpm for 30 minutes before removal of the supernatant. The pellet was air-dried in the dark for 5 minutes and was then re-suspended in 6 µl LSI/WCP hybridization buffer (Vysis/Abbott,). This final mixture represented the probe for hybridization against target metaphase cells.

2.3.3 Hybridization

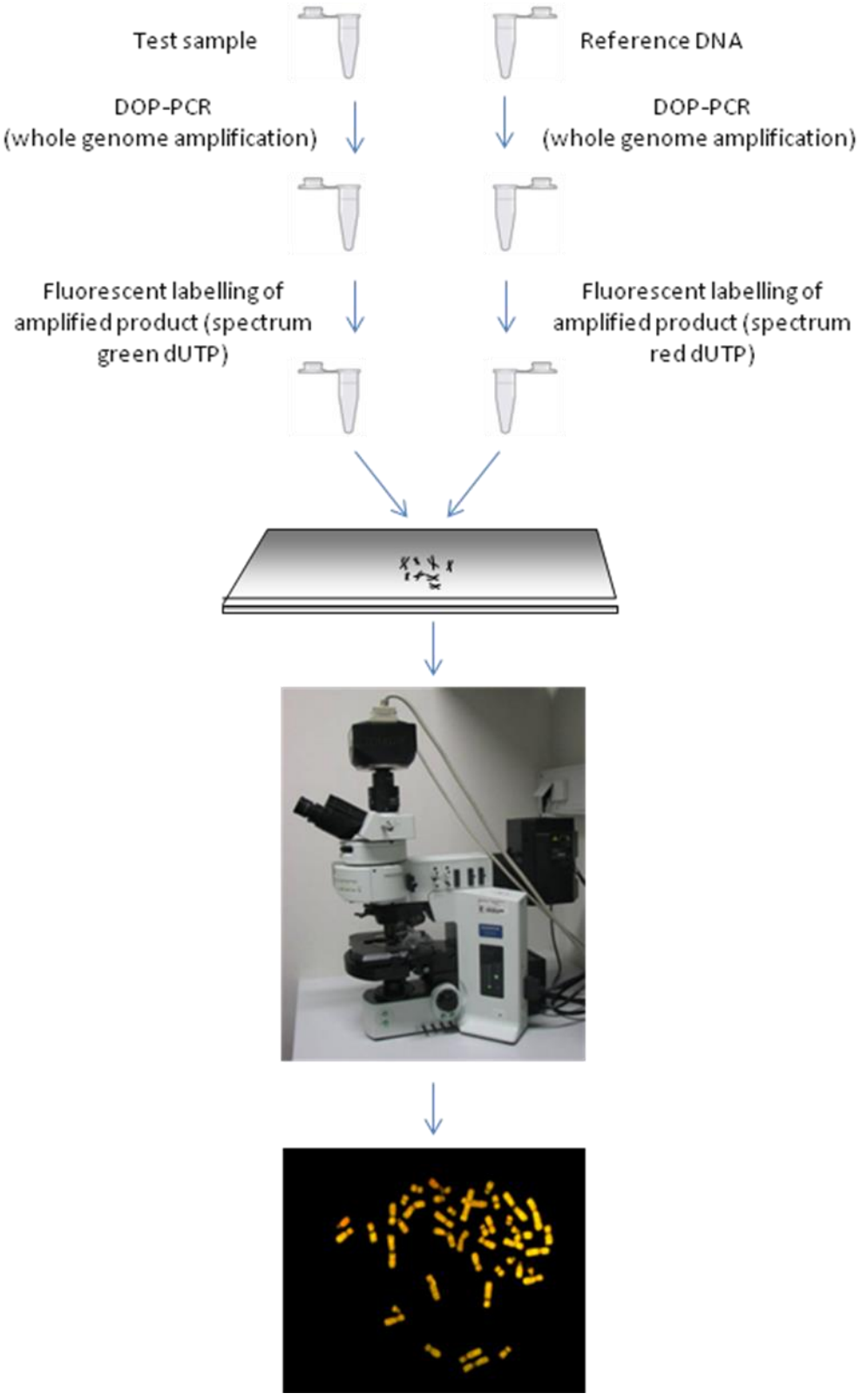
Metaphase target slides (Vysis/Abbott) were denatured in a coplin jar containing 50ml of freshly prepared 70% formamide/2x Saline-Sodium Citrate (SSC) (Appendix A) at 73˚C for 5 minutes. The probes were also denatured at 73˚C for 10 minutes and then applied to the slides. These slides were then incubated for approximately 72 hours at 37˚C in Slide Moat (Boekel scientific), a temperature controlled slide processing system.

2.3.4 Post-hybridization washes, analysis and interpretation

Post-hybridisation washes took place in the dark as follows: 3 minute incubation of the slides in 0.4x SSC/0.3% NP-40 at 73˚C (Appendix A); 3 minute incubation in 2x SSC/0.1% NP-40 (Appendix A) at room temperature.; dehydration through an Ethanol series 70%, 90% and 100% (Appendix A) for 2 minutes each. Finally, slides were dried at room temperature in the dark and then mounted with 10 µl of DAPI-II/Antifade (Vysis/Abbott) under a coverslip.

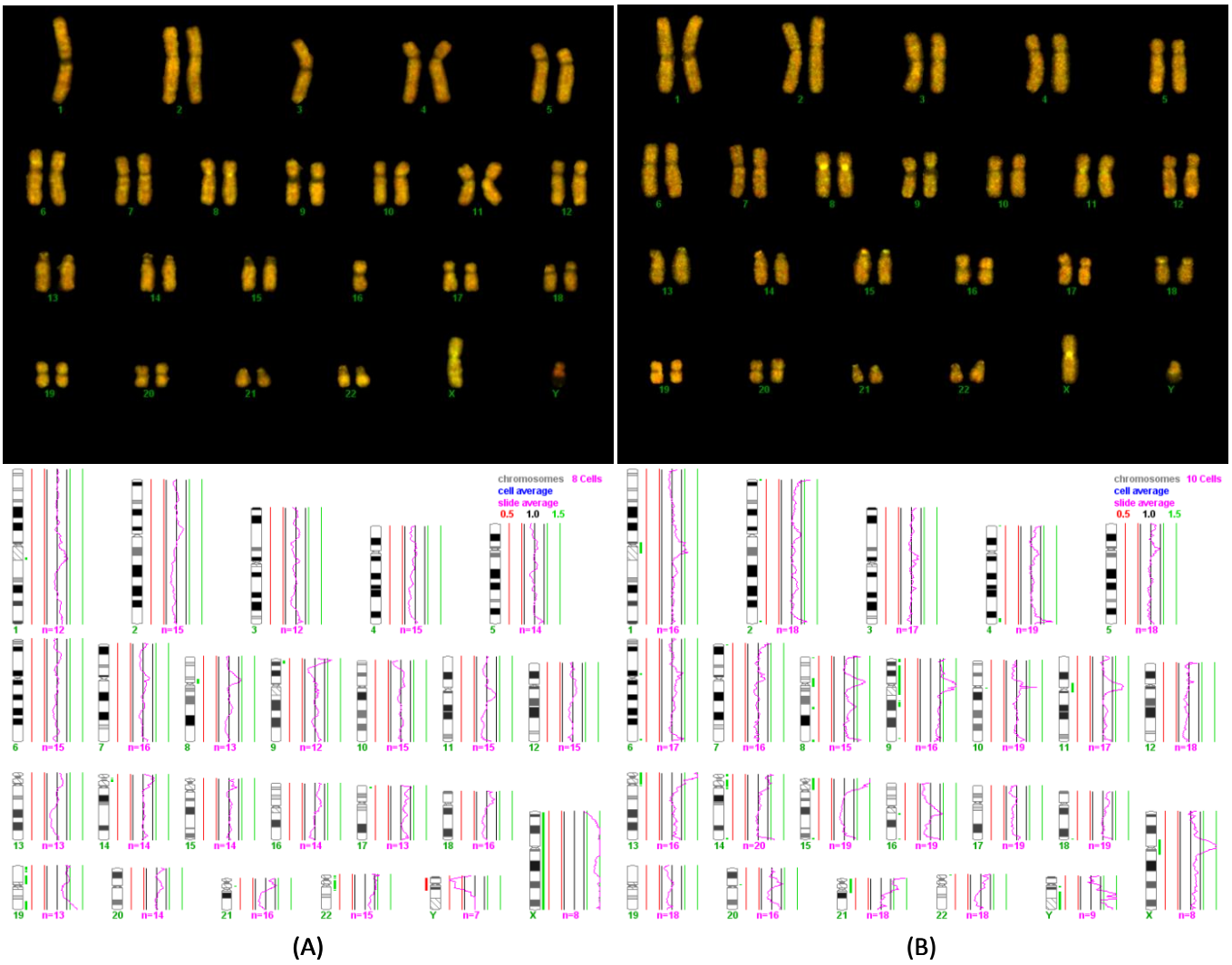
Ten metaphase images were captured per CGH experiment using an Olympus BX61 fluorescence microscope with a cooled charge-coupled device (CCD) system. Analysis and interpretation of these images took place by using the Cytovision CGH software (V3.9, Applied imaging), which converts fluorescence intensities into red:green ratios for each chromosome. Equal sequence copy number between the test and reference DNAs was shown as no significant deviation of the ratio profile from 1:1. A fluctuation of the ratio profile in favour of the red colouration (below 0.80) was considered to indicate test sample under-representation, while a shift of the ratio profile towards the green colouration (above 1.20) was indicative of test sample over-representation. Such fluctuations were, respectively, scored as losses or gains in the test sample, compared with the reference sample. Figure 2-1 illustrates metaphase CGH procedure and Figure 2-2 contains the profile of A) a normal female samples B) a normal male sample.

Figure 2-1: An illustration of metaphase CGH procedure from biopsy to analysis*



*Images in this figure are courtesy of *Samer Alfarawati*

Figure 2-2: Metaphase CGH profile A) Normal female sample B) Normal male sample



(A) (B)

The top images show images of all chromosomes with hybridized red (reference) and green (sample) DNAs. The bottom images represent the red:green flurescence ratio along the length of all chromosomes. The black line in the middle indicates a 1:1 ratio of red:green fluorescence, the green line represents a 1:1.2 ratio (gain) and the red line indicates a 0.8:1 ratio (loss)

2.4 Microarray comparative genomic hybridization (aCGH)

The microarray platform that was used during this project was commercially available (24sure Cytochip, BlueGnome, Ltd) and consisted of DNA clones representing both the small (p) and the long (q) arms of all 24 human chromosomes. This technology is capable of examining the entire chromosome complement for gains as well as losses of material by using a unique BAC pooling strategy which includes the use of 3226 BAC clones (present twice per hybridisation area, i.e. a total of 6452 clones). Specialised computer software—enables robust results to be reported on the chromosome status of single cells or small groups of cells. A high-resolution microarray platform, 24Sure+ (Bluegnome, Cambridge, UK) consisting of almost twice the number of BAC clones was also used, enabling accurate characterization of smaller structural abnormalities.

Similar to metaphase CGH, aCGH combined an initial WGA step, followed by green (Cy3) and red (Cy5) fluorescence labelling, hybridisation, image analysis and interpretation. The molecular karyotypes of unknown "test" samples were assigned by comparing them against normal male (46,XY) or female (46,XX) DNA samples. The majority of reagents used during aCGH were purchased from BlueGnome, Ltd.

2.4.1 WGA via the use of the SurePlex amplification method

Sureplex is a PCR-based WGA which is based on non-enzymatic fragmentation of the DNA followed by ligation of flanking DNA fragments with universal priming sites (adaptors) to form a "library" which is then amplified by the use of primers that bind to these adaptors.

The protocol provided with the Sureplex kit (BlueGnome, Ltd/Rubicon genomics) involved three distinct stages. During the first stage, cell lysis and DNA fragmentation was achieved by adding 3 µl of Cell Extraction Buffer, 4.8 µl of Extraction Enzyme Dilution buffer and 0.2 µl of Cell Extraction Enzyme into each of the tubes containing "test" samples to be examined. Incubation

followed at 75˚C for 10 minutes and 95˚C for 4 minutes. Pre-amplification of the test samples occurred during the second stage by, adding 4.8 µl of Pre-amplification Buffer and 0.2 µl of Pre-amplification enzyme and incubating the tubes at 95˚C for 2 minutes followed by 12 cycles of: 95˚C for 15 seconds, 15˚C for 50 seconds, 25˚C for 40 seconds, 35˚C for 30 seconds, 65˚C for 40 seconds and 75˚C for 40 seconds. The third stage involved the amplification of the test samples and was achieved by mixing 34.2 µl of nuclease-free water and 25 µl Amplification Buffer and 0.8 µl Amplification Enzyme into each tube (total amplification volume of 75 µl). Amplification took place by incubating at 95˚C for 2 minutes followed by 14 cycles of: 95˚C for 15 seconds, 65˚C for a minutes and 75˚C for 1 minute. All incubations took place in an Eppendorf AG 22331 thermal cycler (Germany).

Amplification was confirmed by running 5 µl of each of the test samples on a 1% agarose gel [1 g of agarose (Fisher scientific) dissolved in 100 ml 1x Tris-Borate-EDTA (TBE) buffer (Fisher scientific) plus 9 µl ethidium bromide to label the DNA] in an electrophoresis tank filled with 1x TBE buffer (Fisher scientific) at 200 volts for 10 min, and was seen as smears of DNA fragments, ranging in size between 100-1000 base pairs (average 400/500 bp).

2.4.2 Fluorescence labelling of amplified products

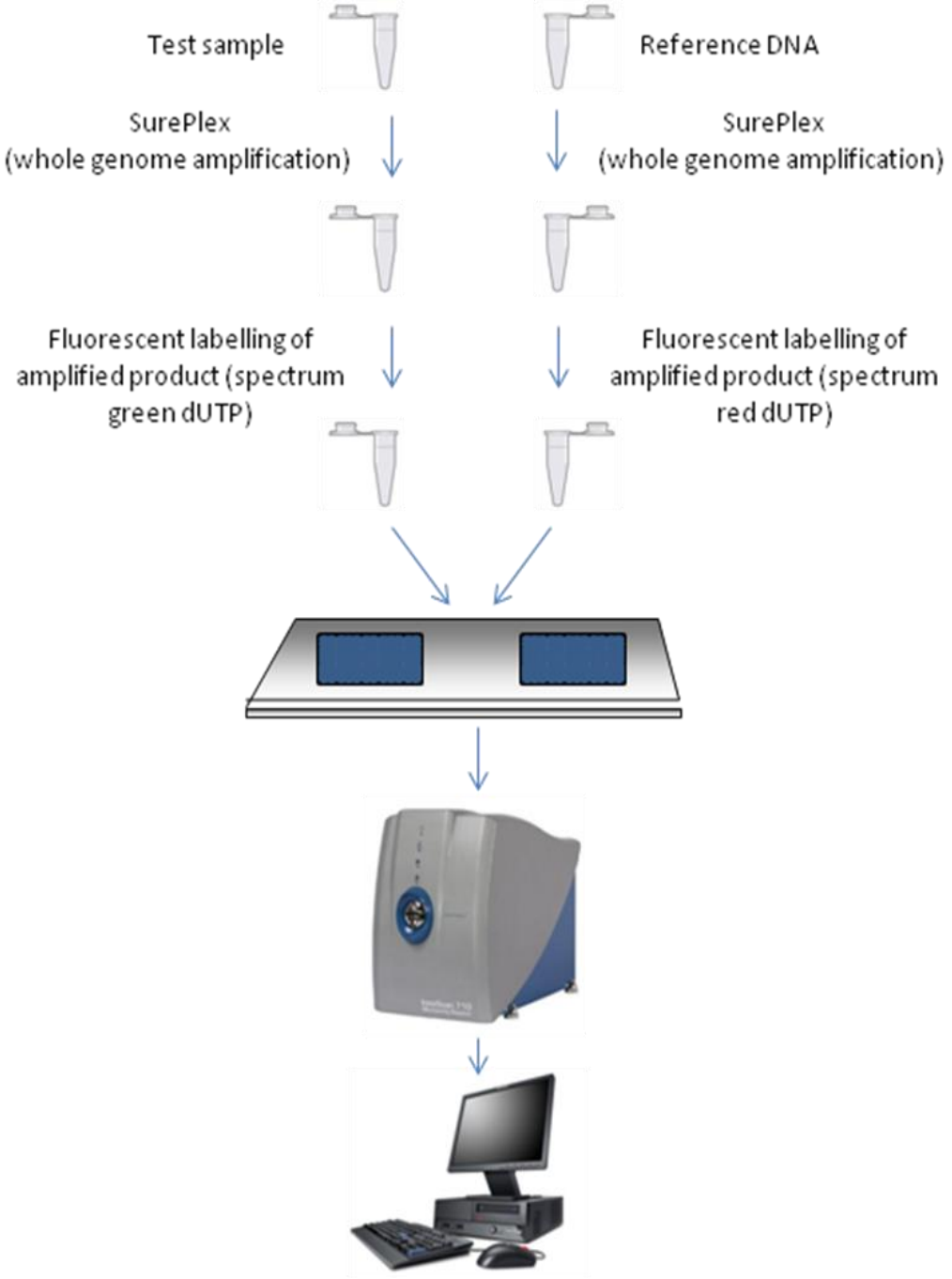
Amplified test sample DNA along with reference DNA (46,XY or 46,XX, provided by Blue-Gnome, Ltd) were labelled using random primers and a Klenow enzyme. The reaction took place in a total volume of 25 µl and included 8 µl of DNA (test or reference), 5 µl of Reaction Buffer, 5 µl of dCTP labelling mix, 5 µl of Primer Solution, 1 µl of Cy3 orCy5 dCTP and 1 µl of Klenow enzyme and was achieved by incubating test and reference DNA samples in a thermal cycler at 37˚C. The incubation time ranged between 90 minutes and 16 hours.

Once the labelling reaction was complete, test and reference DNAs were combined together and mixed with 25 µl of Human Cot-1 DNA (Bluegnome, Ltd). This mix was concentrated via centrifugal evaporation, which took place at 75˚C for 43 minutes. The resulting pellet was re-suspended in 21µl of Hybridization Buffer, and denatured at 75˚C for 10 minutes. The denatured probes were then applied on the microarray slides [24 Sure, or 24 Sure+] (Bluegnome, Ltd) and hybridization took place-overnight at 47˚C in a humidified chamber.

2.4.3 Post hybridization washes, scanning and analysis

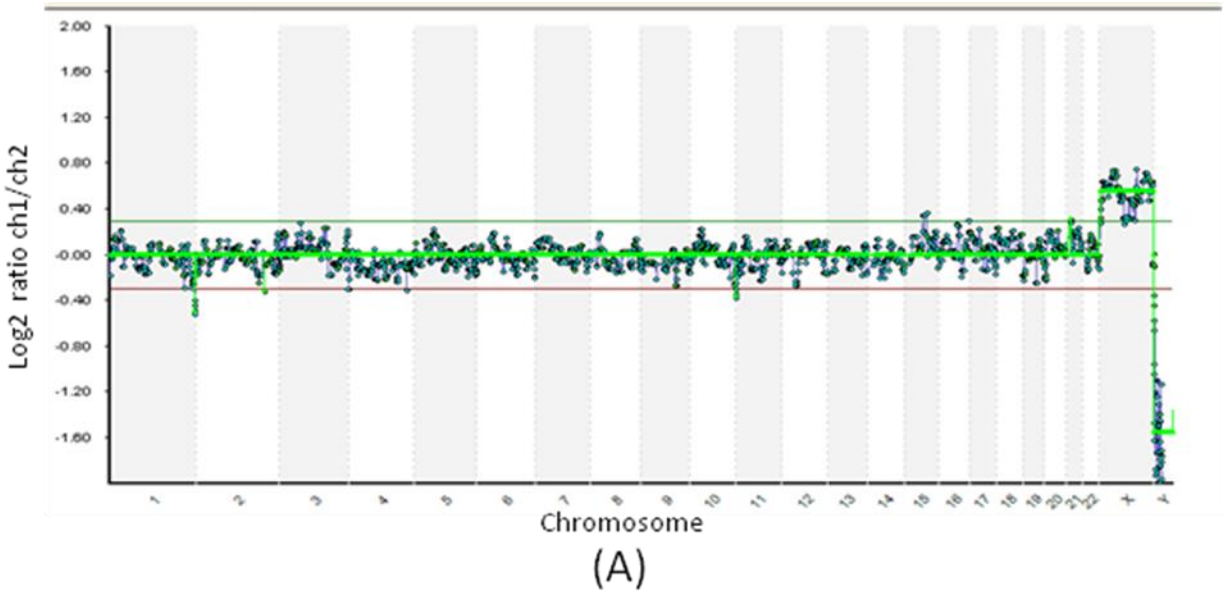
After hybridization, microarray slides were washed as follows: one 10-minute wash in 2x SSC/0.05% Tween-20 (Appendix A) at room temperature, another 10-minute wash in 1x SSC (Appendix A) at room temperature, a 5-minute wash in 0.1x SSC (Appendix A) at 60°C and finally a 1-minute wash at room temperature in the same solution as wash 3. Microarray slides were dried via a 3-minute centrifugation, which also took place at room temperature. Microarrays were scanned using the Innopsys microarray scanner IS700A (Innopsys) and the associated MAPIX software (Innopsys). Scanned images were then analysed using the Bluefuse Multi analysis Software (BlueGnome, Ltd), which is capable of detecting chromosome losses and gains with the use of a 3x SD assessment criteria. Figure 2-3 illustrates aCGH protocol and Figure 2-4 (A) the profile of a female sample (B) the profile of a male sample.

Figure 2-3: An illustration of aCGH procedure from sample biopsy to analysis*

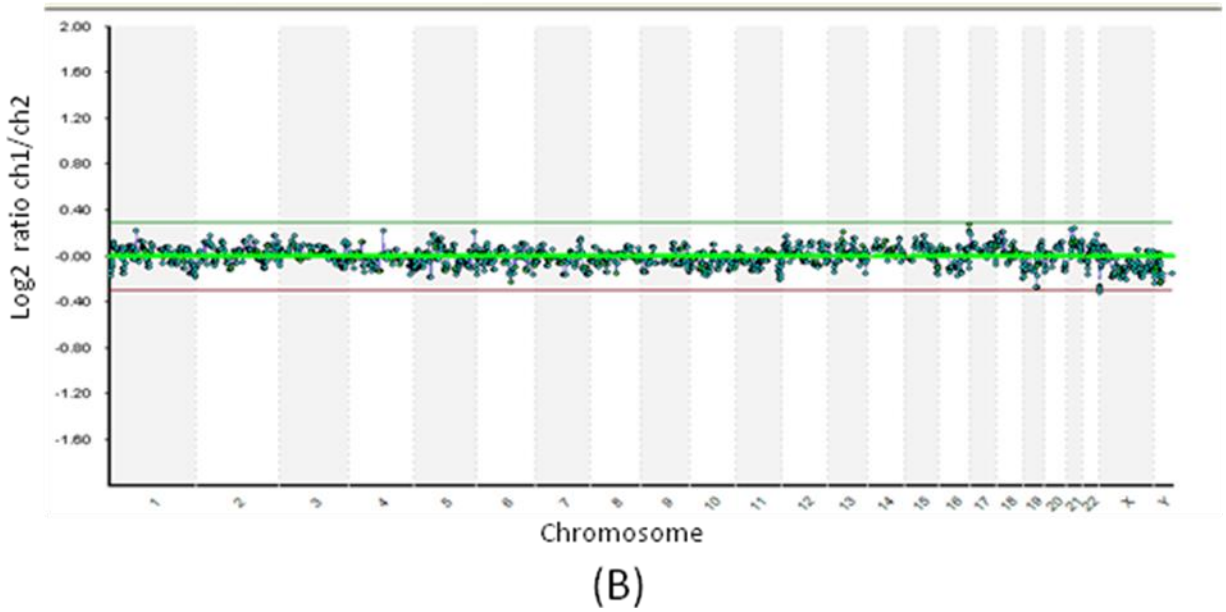


*Images in this figure are courtesy of *Samer Alfarawati*

Figure 2-4: The profile of a normal female sample (A) and a male sample (B)



(A)



(B)

Results of the microarray probes along the length of all chromosmes against a normal male reference

2.5 Materials and methods used in chapter: Comprehensive cytogenetic analysis of human blastocysts:

2.5.1 Participating patients

Twenty couples with normal karyotypes agreed to donate their surplus but potentially transferable unused blastocysts. These couples underwent routine assisted reproductive techniques in four different IVF centres [Colorado centre for reproductive medicine (CCRM), Colorado, USA; London fertility clinic (LFC), London, UK; Oxford fertility unit (OFU), Oxford, UK; IVF Hammersmith, London, UK], and the average female age was 36 years (age range 24–42 years). Details regarding each patient and the analysis methods applied to the analysis of the embryos are present in Table 2-2.

2.5.2 Metaphase CGH

Experienced embryologist performed the trophectoderm (TE) cells biopsied from all fifty-two embryos were tested using metaphase CGH in our laboratory following the protocol described in section 2.3.

2.5.3 Microarray CGH

Experienced embryologist performed the second TE biopsy for twenty of the 52 blastocysts, and the samples obtained were tested using the 24Sure microarray platform in our laboratory (BlueGnome Ltd) following protocol described in 2.4.

2.5.4 Fluorescent *in situ* hybridization (FISH)

Fluorescent *in situ* hybridization is a molecular cytogenetic technique which uses fluorescently-tagged probes capable of hybridising onto specific areas on chromosomes. During this project, FISH was used in order to examine the chromosome content of either single blastomeres, or entire embryos. Experienced embryologist from all four clinics spread and fixed embryonic cells onto microscope slides, and the procedure is described below.

Table 2-2: Patient age, number of embryos tested and different test used to examine the blastocyst

Patient	Female age	Embryo no.	CGH	aCGH	Chromosomes tested by FISH		
					1st round	2nd round	3rd round
1	24	1	Yes	Yes	13, 16, 18, 21, 22	15,17,X,Y	N/A
2	25	1	Yes	Yes	13, 16, 18, 21, 22	15,17,X,Y	N/A
3	30	1	Yes	Yes	13, 16, 18, 21, 22	15,17,X,Y	N/A
		2	Yes	Yes	13, 16, 18, 21, 22	15,17,X,Y	N/A
		3	Yes	Yes	13, 16, 18, 21, 22	15,17,X,Y	N/A
		4	Yes	Yes	13, 16, 18, 21, 22	15,17,X,Y	N/A
4	32	1	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	N/A
		2	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	N/A
		3	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	N/A
		4	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	N/A
5	32	1	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	12
		2	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	N/A
		3	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	N/A
		4	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	N/A
6	33	1	Yes	Yes	13, 16, 18, 21, 22	15,17,X,Y	N/A
		2	Yes	Yes	13, 16, 18, 21, 22	15,17,X,Y	N/A
7	34	1	Yes	Yes	13, 16, 18, 21, 22	15,17,X,Y	N/A
		2	Yes	Yes	13, 16, 18, 21, 22	15,17,X,Y	N/A
		3	Yes	Yes	13, 16, 18, 21, 22	15,17,X,Y	N/A
		4	Yes	Yes	13, 16, 18, 21, 22	15,17,X,Y	N/A
8	35	1	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	N/A
		2	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	4
		3	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	6
9	37	1	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	N/A
		2	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	N/A

10	38	1	Yes	Yes	13, 16, 18, 21, 22	15,17,X,Y	N/A
		2	Yes	Yes	13, 16, 18, 21, 22	15,17,X,Y	N/A
11	39	1	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	N/A
		2	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	N/A
		3	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	N/A
12	39	1	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	N/A
		2	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	N/A
13	39	1	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	N/A
		2	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	N/A
		3	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	N/A
		4	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	8
		5	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	6
14	39	1	Yes	Yes	13, 16, 18, 21, 22	15,17,X,Y	N/A
		2	Yes	Yes	13, 16, 18, 21, 22	15,17,X,Y	N/A
15	40	1	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	N/A
		2	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	N/A
		3	Yes	Yes	13, 16, 18, 21, 22	15,17,X,Y	20
16	40	1	Yes	Yes	13, 16, 18, 21, 22	15,17,X,Y	N/A
		2	Yes	Yes	13, 16, 18, 21, 22	15,17,X,Y	6
		3	Yes	Yes	13, 16, 18, 21, 22	15,17,X,Y	N/A
17	41	1	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	N/A
18	41	1	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	19 and 20
		2	Yes	No	13, 16, 18, 21, 22	15,17,X,Y	19 and 20
19	42	1	Yes	No	13, 16,18, 21, 22	15,17,X,Y	3, 9, 11
		2	Yes	No	13, 16,18, 21, 22	15,17,X,Y	9, 14
20	42	1	Yes	No	13, 16,18, 21, 22	15,17,X,Y	7, 20
		2	Yes	No	13, 16,18, 21, 22	15,17,X,Y	7, 12

2.5.4.1 Sample spreading and fixation

The remainder of each of the 52 embryo was spread by experienced embryologists from participating clinics on microscope slides using either the methanol/acetic acid (Griffin et al., 1992; Munné et al., 1993) or Tween-20/HCl (Harper et al, 1994) fixation techniques. For the methanol/acetic acid method, embryos were exposed to a hypotonic solution [0.075 M KCl with 0.6% bovine serum albumin (BSA)] for 2 minutes, and then transferred into a drop of hypotonic solution on the microscope slide. As soon as the hypotonic solution was beginning to dry, a single drop of fixative methanol/acetic acid solution was added over the blastomere, so as to remove cytoplasm and fix the nucleus.

For the Tween-20/HCl method, the embryos were fixed onto poly-L-lysine coated slides. Specifically, embryos were washed in phosphate-buffered saline (PBS), and then transferred to a drop of spreading solution (0.01 N HCl, 0.1% Tween-20) [Appendix A] on the slide. In order to remove cytoplasmic remnants and make the nucleus accessible for the DNA probes, slides were incubated in 100 µg/ml pepsin in 0.01N HCl (Appendix A) for 20 minutes at 37°C, and then re-fixed in 1% paraformaldehyde (Appendix A) at 4°C for 10 minutes.

2.5.4.2 FISH probes used on blastocysts

Fixed blastocysts were analysed using FISH in two or three rounds in our laboratory throught the entire procedure. A control slide of fixed lymphocyte nuclei obtained from a male of normal karyotype (46,XY) was processed along with the embryo slides to allow monitoring of probe hybridization efficiency. Chromosomes 13, 15, 16, 17, 18, 21, 22, X and Y were examined in all blastocysts. Additionally, specific chromosomes that metaphase CGH or aCGH results suggested to be aneuploid were also tested in the affected embryos. The extra chromosomes tested were: 3, 4, 6, 7, 8, 9, 11, 12, 19 and 20 (Table 2-2).

The MultiVysion PB probe panel [for chromosomes 13, 16, 18, 21 and 22, (Vysis/Abbott)] was used in the first round of FISH for all embryos tested and the 4CC custom probe panel [for chromosomes 15, 17, X and Y (Vysis/Abbott)] was used in the second round. Eleven blastocysts underwent a third round of FISH and 1-3 chromosomes were tested (table 3-1). The probes used to examine chromosomes not covered in the panels mentioned above are included in Table 2-2. Details of all FISH probes used are presented in Table 2-3.

Table 2-3: Details about probes used in different FISH experiments

Chromosome	Probe name	Probe location	Probe type	Fluorophore
3	D3Z1	3p11.1–q11.1	Centromeric	Spectrum orange
4	CEP 4	4p11–q11	Centromeric	Spectrum aqua
6	D6Z1	6p11–q11.1	Centromeric	Spectrum aqua
7	D7Z1	7p11.1–q11.1	Centromeric	Spectrum aqua
8	D8Z2	8p11.1–q11.1	Centromeric	Spectrum aqua
9	CEP 9	9p11.1–q11.1	Centromeric	Spectrum aqua
11	D11Z1	11p11.1–q11.1	Centromeric	Spectrum aqua
12	D12Z3	12p11.1–q11.1	Centromeric	Spectrum green
13	Vysis LSI 13	13q14.1–q14.3	Locus specific	Spectrum red
14	TelVysion 14q	D14S1420	Locus specific	Spectrum orange
15	D15Z4	15p11.1–q11.1	Centromeric	Spectrum orange
16	D16Z3	16q11.2	Centromeric	Spectrum aqua
17	D17Z1	17p11.1–q11.1	Centomeric	Spectrum aqua
18	D18Z1	18p11.1–q11.1	Centromeric	Spectrum blue
19	TelVysion 19q	D19S238E	Sub-telomeric	Spectrum orange
20	D20Z1	18p11.1–q11.1	Centromeric	Spectrum orange
20	TelVysion 20p	20pTEL18	Sub-telomeric	Spectrum green
21	Vysis LSI 21	21q22.13–q22.2	Locus specific	Spectrum green
22	Vysis LSI22BCR	22q11.2	Locus specific	Spectrum gold
X	CEP X DXZ1	Xp11.1–q11.1	Centromeric	Spectrum green
Y	CEP Y DYZ1	Yq12	Centromeric	Spectrum blue

2.5.4.3 Hybridization, post-hybridization washes and analysis

Probe mixtures were applied to the slides and probe/sample co-denaturation was performed at 73°C for 5 minutes in ThermoBrite (Abbott molecular), a temperature controlled slide processing system. Hybridization took place at 37°C overnight in ThermoBrite. Post-hybridization washes to remove unattached probes took place in 0.7x SSC/0.3% NP-40 at (Appendix A) 72°C for 4 minutes, and then in 0.7x SSC/0.3% NP-40 (Appendix A) at room temperature for 1 minute.

Slides were examined under an Olympus BX61 fluorescence microscope with a CCD system. All scoring decisions were taken by directly viewing signals under the microscope. Scoring criteria, as described by Munné et al (1998b), were used to characterise the chromosomal content of embryonic blastomeres. These criteria were as follows:

- Split signal: when the nucleus is in interphase, some homologues would consists of 2 chromatids which leads to 2 probe signals very close together and each one is smaller in size than a single regular signal domain.
- A signal rarely splits into two signals separated more than two signal domains apart.
- Diffused signal: a stretched non-interrupted signal that usually appears in very decondensed nuclei mainly affecting centromeric probes.

2.5.4.4 Embryo Classification

Results obtained from FISH, CGH and aCGH were combined and classification of embryos took place according to criteria published by Delhanty et al. (1997). An outline of these classification criteria are shown in Table 2-4.

Table 2-4: Embryo classification according to chromosome complement

Classification	**Chromosome constitution**
Normal	All nuclei uniformly diploid
Abnormal (non-mosaic)	All nuclei uniformly aneuploid
Abnormal (mosaic diploid-aneuploid)	Two or more distinct cell lines: one euploid and the other(s) aneuploid
Abnormal (mosaic aneuploid)	Two or more distinct aneuploid cell lines, no chromosomally normal cells
Chaotic	Each nuclei showing different chromosome aneuploidies

2.6 Materials and methods used in chapter: The effect of aneuploidy on embryo morphology and development to the cleavage and blastocyst stage

2.6.1 Patient details

The embryos examined during this part of the project were generated by 229 patients undergoing assisted reproductive treatment combined with preimplantation genetic screening (PGS) at 8 different IVF clinics in the UK and the USA. The average maternal age was 38.8 years (age range 27 –47 years). This patient group was further divided into 2 sub-groups depending on the time their embryos spend in culture; the first group included 67 patients with an average maternal age of 39.3 years (age range 27-45 years) who had their 436 embryos cultured until day-3 (cleavage-stage) when biopsy of a single blastomere took place. Normal embryos were transferred either on day-4 or day-5 of development, depending on the IVF clinic. The second group included 162 patients with an average maternal age of 38.6 years (age range 29-47 years) whose 961 embryos were cultured until day 5 (blastocyst stage) when biopsy of a small group of trophectoderm (TE) cells took place. Normal blastocysts were either transferred on day-6, or were frozen via vitrifcation and transferred in a subsequent cycle.

2.6.2 Cleavage stage and blastocyst stage embryo scoring

Embryo morphology was assessed and recorded on days-3 and/or 5-6 post-oocyte retrieval by experienced embryologist in all participating clinics. Previously published morphological criteria were used for this purpose (Gardner and Schoolcraft, 1999; Scott et al., 2007) and are described in Tables 2-5, 2-6 and 2-7 below.

Table 2-5: Morphological criteria employed during day-3 embryo scoring

Embryo grade	Interpretation
1	6-8 blastomeres of equal size and no visible fragmentation or multinucleation
2	6-8 blastomeres of even or uneven shape with less than 10–20% fragmentation, or multinucleation
3	Less than 6 cells of equal size and no fragmentation or multinucleation
4	More than 20% fragmentation or very uneven, atypical cell size
5	Arrested or totally fragmented embryo

Table 2-6: Morphological criteria employed during day-5 embryo scoring, embryos expansion

Blastocyst expansion grade	Interpretation
6	Hatched blastocyst- the blastocyst has completely escaped the zona pellucida
5	Hatching blastocyst- trophectoderm (TE) cells have begun to herniate through the zona pellucida
4	Expanded blastocyst- the blastocoel is now larger than the early embryo, and the zona pellucida shows significant thinning
3	Full blastocyst- the blastocoel fills the embryo completely
2	Blastocyst- the blastocoel occupies more than one-half of the volume of the embryo
1	Early blastocyst- the blastocele accounts for less than one-half of the volume of the embryo

Table 2-7: Morphological criteria employed during day-5 embryo scoring, inner cell mass and trophectoderm quality

ICM grade	Interpretation	TE grade	Interpretation
A	Tightly packed with many cells	A	Many cells forming a cohesive epithelium
B	Loosely grouped with several cells	B	Few cells forming a loose epithelium
C	Very few cells	C	Very few large cells.

2.6.3 Test methods used to analyze the embryos

Metaphase CGH and aCGH were the methods used to investigate the chromosomal status of these embryos. All embryos biopsied at the cleavage-stage and 141 blastocysts were tested by aCGH (as described in 2.4), the remaining blastocysts (820) were analysed by metaphase CGH (as described in 2.3). All embryos were analysed in our laboratory.

2.7 Materials and methods used in chapter: Preimplantation Genetic Diagnosis of chromosome rearrangements using comprehensive molecular cytogenetic methodology

2.7.1 Patient and embryo details

The chromosomal rearrangements assessed during this part of the research project had already been defined by accredited cytogenetic laboratories using standard chromosome banding techniques.

A total of 48 PGD cycles for 39 couples carrying a variety of chromosome rearrangements were performed. The average female age was 35.7 years (age range 29-43 years) and the chromosome rearrangements included 16 Robertsonian translocations, 21 reciprocal translocations and 3 inversions. For one couple, the male partner was a carrier of a reciprocal translocation and the female partner was a carrier of an inversion. Patient details, developmental stage at which biopsy was performed and associated clinical outcomes are summarized in Table 2-8.

Sixteen of the cases involved biopsy of ~5 trophectoderm cells (TE) from 93 blastocysts; three cases assessed had first and second PBs biopsied from 25 fertilised oocytes (zygotes) (25 first and 25 second PBs); twenty nine cases involved biopsy of blastomeres from 46 cleavage-stage embryos (single cell biopsy). All biopsies involved breach of the zona pellucida with a laser, regardless of the embryonic stage being tested and was performed by trained embryologists in different IVF clinics where patients were receiving their treatment. Specific details of stimulation, biopsy and embryo culture varied between the referring clinics, but did not differ significantly from methods considered to be standard.

Table 2-8: Summary of investigated samples and associated clinical outcomes

Patient number	Case number	Age	Karyotype of rearrangement carrier	Stage of biopsy	Normal/ balanced	Abnormal	Outcome
1	**1**	29	46,XY,t(17;19)(q25.3;q13.1)	Cleavage	1	6	Not known
	2				2	9	Not known
2	**3**	30	46,XY,t(16;17)(q11;p13.3)	Cleavage	0	5	No transfer
3	**4**	31	45,XY,der(13;14)(q10;q10)	Blastocyst	4	9	Not pregnant
	5			Blastocyst	6	3	Delivered
4	**6**	31	46,XY,t(1;2)(q23.1;q35)	Blastocyst	0	3	No transfer
	7			Cleavage	0	5	No transfer
5	**8**	31	45,XY,der(14;21)(q10;q10)	Blastocyst	1	1	Not pregnant
6	**9**	32	45,XY,der(13;14)(q10;q10)	Cleavage	1	3	No transfer – Normal embryo arrested
7	**10**	32	46,XY,t(1;3)(q31;p13)	Blastocyst	3	4	Not known
8	**11**	33	45,XY,der(14;21)(q10;q10)	Cleavage	1	1	Not pregnant
9	**12**	33	46, XY, t(5;15)(q13.1;q21.2)/ 46,XX,inv(3)(q25.1;q26.2)	Cleavage	0	8	No transfer
10	**13**	33	46,XY,t(11;22)(q23;q11.2)	Blastocyst	3	6	Delivered
11	**14**	34	45,XY,der(13;15)(q10;q10)	Blastocyst	0	11	No transfer
12	**15**	34	45,XY,t(13;15)(q21.3;q11.2)	Cleavage	0	3	No transfer
13	**16**	34	45,XY,der(13;15)(q10;q10)	Cleavage	2	5	Not known
14	**17**	34	46,XX,t(17;18)(p11.2;p11.2)	Cleavage	0	3	No transfer
15	**18**	34	46,XX,t(8;9)(p12;q31)	Blastocyst	2	3	Not known
16	**19**	35	46,XY,t(1;8)(q25.3;p11.2)	Cleavage	1	1	Delivered
17	**20**	35	46,XX,t(10;16)(q11.2;p11.2)	Cleavage	3	2	Biochemical pregnancy
18	**21**	35	46,XX,t(1;2)(q24;p21)	Cleavage	0	3	No transfer
	22	36		Blastocyst	2	0	Not pregnant
19	**23**	35	45,XY,der(13;14)(q10;q10)	Cleavage	1	6	Not pregnant
20	**24**	35	46,XX,t(1;5)(p36.1;q33)	Blastocyst	0	5	No transfer
	25				1	3	Ongoing pregnancy
21	**26**	36	46,XY,inv(8)(p21q24.1)	Blastocyst	2	1	Delivered
22	**27**	36	46,XY,t(10;17)(q21.2;p11.2)	Cleavage	0	3	No transfer

23	**28**	36	45,XY,der(13;14)(q10;q10)	Cleavage	0	2	No transfer
24	**29**	36	46,XY,t(7;18)(p15.3;q12.2)	Cleavage	1	3	Not known
25	**30**	36	45,XY,der(13;14)(q10;q10)	Cleavage	1	4	Not known
26	**31**	36	45,XX,der(13;14)(q10;q10)	Cleavage	0	8	No transfer
27	**33**	37	45,XX,der(13;21)(q10;q10)	Cleavage	1	5	Not pregnant
	34				1	4	Not known
28	**35**	37	46,XY,t(1;3)(q31;p13)	Cleavage	0	9	No transfer
29	**36**	37	45,XY,der(13;14)(q10;q10)	Cleavage	0	6	No transfer
30	**37**	38	45,XY,der(14;21)(q10;q10)	Cleavage	0	2	No transfer
31	**38**	39	45,XY,der(14;21)(q10;q10)	Cleavage	1	7	Not known
32	**39**	39	46,XY,t(17;21)(q21;q22)	Cleavage	0	2	No transfer
33	**40**	40	46,XY,t(1;15)(q21p11.2)	Cleavage	1	6	Not pregnant
34	**41**	40	46,XX,t(7;10)(p11.2;p11.23)	Cleavage	1	2	Not pregnant
35	**42**	41	46,XY,t(5;7)(q23.2;p14)	Blastocyst	1	2	Normal embryo arrested- No transfer
36	**43**	41	45,XX,der(13;14)(q10;q10)	Blastocyst	0	5	No transfer
37	**44**	42	45,XX,der(13;14)(q10;q10)	Zygote (polar bodies biopsied)	1	8	No transfer – Normal embryo arrested
	45			Zygote (polar bodies biopsied)	0	8	No transfer
37	**46**	42	45,XX,der(13;14)(q10;q10)	Blastocyst	0	3	No transfer
38	**47**	42	46,XX,t(4;11)(q24;q11)	Cleavage	0	5	No transfer
39	**48**	43	46,XX,inv(5)(p13.1-q13.3)	Zygote (polar bodies biopsied)	2	5	Not pregnant
	49	43	46,XX,inv(5)(p13.1-q13.3)	TE biopsy	0	2	No transfer

2.7.2 Methods used for embryo analysis

Metaphase and aCGH were the methods used to investigate the chromosomal status of fertilised oocytes and embryos. All cleavage-stage embryos and 47 blastocysts were examined by aCGH, and the rest of the blastocysts (46) along with polar body samples were analyzed by metaphase CGH. All analysis procedures involving CGH and aCGH was performed in our laboratory.

2.7.2.1 Metaphase CGH

Twenty-five first and second polar bodies and forty-six TE cells were analysed using metaphase CGH following the protocol described in section 2.3 (Metaphase CGH).

Previous studies have shown that metaphase CGH, is able to detect unbalanced fragments as small as 10 Mbp (Wells and Delhanty, 2000).

2.7.2.2 Microarray CGH

One hundred and fifty-nine blastomeres and forty-seven TE samples were tested using aCGH (BlueGnome Ltd) [as explained in details in section 2.4, titled Microarray CGH]. The aCGH resolution ranged between 2-10 Mbp depending on the type of used microarray platform. Lower resolution microarray slides were used during the PGD of Robertsonian translocations (24Sure, BlueGnome), whereas higher resolution slides were employed for the PGD of reciprocal translocations and inversions (24Sure+, BlueGnome).

2.7.2.3 FISH follow up analysis

Embryos from patients 3, 8, 10 and 11 which were diagnosed as aneuploid following metaphase CGH or aCGH analysis and those that were diagnosed euploid following analysis but that arrested were spread on microscope slides and fixed using Tween-20/HCl fixing method (explained in section 2.5.4.1) by trained embryologists in IVF clinics and tested with FISH using probes for chromosomes involved in the rearrangement plus probes for chromosomes 13, 16, 18, 21 and 22 which was used for all re-analysed embryos. FISH procedure and analysis was performed in our laboratory. Chromosome involved in the rearrangement and that were tested in embryos from different patients were chromosome 14 for patients 3 and 8, chromosome 11 for patient 10 and chromosome 15 for patient 11. Table 2-9 lists the probes used for embryo re-analysis.

Table 2-9: FISH probes used for embryo re-analysis

Chromosome	Probe name	Probe location	Probe type	Fluorophore
11	D11Z1	11p11.1–q11.1	Centromeric	Spectrum aqua
11	TelVysion 11q	D11S1037	Sub-telomeric	Spectrum orange
13	Vysis LSI 13	13q14.1–q14.3	Locus specific	Spectrum red
14	TelVysion 14q	D14S1420	Locus specific	Spectrum orange
15	D15Z4	15p11.1–q11.1	Centromeric	Spectrum orange
16	D16Z3	16q11.2	Centromeric	Spectrum aqua
18	D18Z1	18p11.1–q11.1	Centromeric	Spectrum blue
21	Vysis LSI 21	21q22.13–q22.2	Locus specific	Spectrum green
22	Vysis LSI22BCR	22q11.2	Locus specific	Spectrum gold

2.7.3 Control samples

For evaluation of the presence of an ICE a robust control group was created for each patient by careful matching with multiple chromosomally normal couples of identical female age, having treatment at the same clinic during the same period of time and using the same diagnostic test (i.e. CGH or aCGH) to assess oocytes or embryos at an identical stage of development (i.e. polar bodies, blastomeres or trophectoderm). The patients in the control group were having chromosome testing as part of an IVF cycle, hoping to improve the selection of viable embryos for transfer (PGS). The control group consisted of 4,840 samples derived from 606 (414 day 3, patients, 170 day 5, 22 PB) (analysis of >199,000 chromosomes). The chromosomes involved in the rearrangement in each patient were excluded from analysis in the corresponding control group, so the focus remained on chromosomes uninvolved in the rearrangement

2.8 Materials and methods used in chapter: The cytogenetics and implantation ability of embryos generated by an infertile population

2.8.1 Patient groups

The samples analysed in this part of the project were generated from 300 patients who underwent assisted reproductive treatment coupled with PGS for three different indications: advanced maternal age (AMA) (35 years or more), recurrent miscarriages (RM) [2 or more previous abortions], and repeated implantation failure (RIF) [at least three failed IVF attempts]. The average maternal age was 39.2 years (age range 27 – 47 years). Table 2-10 includes the number of patients who had their IVF treatment in each participating clinic along with the number of embryos assessed from each clinic.

Table 2-10**:** A list of IVF clinics and number of patients who had their treatment in each clinic

IVF clinic	**Number of patients**	**Number of embryos biopsied**	
		Day-3	**Day-5**
ARGC, UK	2	2	7
Birmingham Women's fertility centre, UK	4	66	0
Bridge Fertility Centre, UK	19	45	26
CRGH, UK	3	19	0
Colorado Centre for Reproductive medicine USA	126	0	783
CRM, UK	9	68	0
IVF Hammersmith, UK	21	42	45
London Fertility Clinic, UK	29	201	29
Lister Fertility Clinic, UK	30	148	54
London Women's clinic, UK	17	68	13
Oxford Fertility Unit, UK	40	130	99

2.8.1.1 Patient group 1: Advanced maternal age

This group included a total of 177 patients with an average maternal age of 40.6 years (age range 35-47 years) and was further divided into two different sub-groups, according to the examined developmental stage. The first sub-group consisted of 73 patients of average maternal age of 40.8 years (age range 35-45 years) who had their 491 embryos cultured until day 3 (cleavage stage) when biopsy of a single blastomere took place. The second sub-group consisted of the remaining 104 patients, whose average maternal age was 40.4 years (age range 35-47 years). The 520 embryos generated by this second sub-group were cultured until day 5 (blastocyst stage), at which point 3-10 cells (average 5 cells) from the external trophectoderm layer were biopsied. Patient details are presented in tables D-1 and D-2 in Appendix D.

2.8.1.2 Patient group 2: Recurrent miscarriages

This group included 64 patients with average maternal age of 37 years (age range 30-45 years). Similarly to the AMA patients, the RM patients were grouped into separate categories, depending on the developmental stage investigated. The cleavage stage sub-group included 160 embryos generated by 23 patients with average maternal age of 37.9 years (age range 31-45 years). The blastocyst stage consisted of 312 embryos and 41 patients whose average maternal age was 36.6 years (age range 30-43 years). Patient details are shown in tables D-3 and D-4 in Appendix D.

2.8.1.3 Patient group 3: Repeated implantation failure

This group included 59 patients with average maternal age 37.3 years (age range 27 – 44 years). Of the two individual sub-groups, the first (cleavage stage) included 18 patients with average maternal age 36.8 years (age range 28-44 years) and 144 embryos. The second (blastocyst) sub-group included 41 patients with (average maternal age of 37.5 years, age range 27-44 years) and 265 embryos. Patient details in this category are shown in tables D-5 and D-6 in Appendix D.

2.8.2 Expected miscarriage rate

The expected pregnancy loss rate for the recurrent miscarriage group was calculated on the basis of the individual's history according to the study by Brigham et al (1999), where depending on the female's age and number of previous miscarriages, a logistic regression was conducted and a formula was derived to predict the probability of achieving a successful pregnancy, the formula is:

$$\text{Ln}(P/[1-P]) = 2 - 0.0828 \times (\text{age} - \text{Mean of age}) - 0.2467 \times (\text{Number of previous miscarriages})$$

P: predicted probability of a successful pregnancy.

The probability of a successful pregnancy according to this formula was calculated for completed cases where the outcome of the treatment was known. The cumulative mean value of those individual patients represents the predicted probability of ongoing pregnancy amongst this patient group. This predicted pregnancy loss rate can be compared to the observed pregnancy loss in this project.

2.8.3 Cytogenetic methodology

Both metaphase and aCGH were used to investigate the chromosomal status of the embryos by this infertile population. All embryos biopsied at the cleavage-stage and 171 blastocysts were tested by aCGH (as described in 2.4), and the rest of the blastocysts (913) were analyzed by metaphase CGH (as described in 2.3).

2.9 Statistical analysis

Statistical analysis was carried out, whenever appropriate, for samples included in the different studies described in this work. Depending on the nature of the data being analysed, different statistical tests were used including: Fisher's Exact Test (GraphPad, USA), Chi-Square Test (GraphPad, USA)

3. Comprehensive cytogenetic analysis of human blastocysts

3.1 Aims

Chromosome abnormalities that arise during mitotic divisions after fertilization lead to chromosomal mosaicism in human preimplantation embryos. Previous investigation of this phenomenon has involved the use of fluorescence *in situ* hybridization (FISH) in cleavage stage (Delhanty et al, 1997; Munné et al, 2002; Katz-Jaffe et al, 2004) and blastocyst stage embryos (Evsikove and Verlinsky, 1998; Magli et al, 2000; Bielanska et al, 2002; Santos et al, 20100). FISH offers the possibility of analysing multiple cells from individual embryos but only for a limited number of chromosomes.

The main aim of this part of the research project was to investigate whether embryos that reach the final stages of embryonic development would have lower (if any) rates of chromosome abnormalities? Moreover, we aimed to test the hypothesis that chromosome abnormalities of post-zygptic origin leading to mosaicism are less frequent at the blastocyst stage?

Three different molecular cytogenetic methods were employed for this purpose, namely FISH, metaphase CGH and aCGH. A secondary aim was the evaluation/validation of the 24sure microarray platform (BlueGnome, Ltd) and its ability to detect chromosome errors in trophectoderm (TE) samples biopsied from human blastocysts.

3.2 Results

3.2.1 Cytogenetic findings of blastocysts examined by CGH and FISH

Thirty-two blastocysts donated by 12 of the participating couples (average maternal age 38.3 years) underwent a single TE biopsy, followed by CGH. The remainder each embryo was fixed onto a microscope slide and examined by a two- or three-round FISH (as mentioned in Table 2-3 above). The mean number of cells assessed by FISH was 14 per blastocyst, while the number of cells tested by CGH was between 5 and 10 cells. Both FISH and CGH yielded results for all analysed samples. The molecular karyotypes, assigned based upon the combined results of CGH and FISH, are listed in Table 3-1. Discrepancies between results obtained by FISH and by CGH were found in 2 (6%) of the 32 embryos (13-1 and 18-1), while the other 30 (94%) embryos gave concordant results for both methods (7 normal and 23 aneuploid).

As far as the 2 embryos with the discrepancies are concerned, the CGH result obtained for blastocyst 13-1 was 46,Y0,+22, whereas FISH showed that 6/16 cells had a normal copy number for all examined chromosomes (i.e. 13, 15, 16, 17, 18, 21, 22, X and Y) and 10/16 cells had lost one of the two copies of chromosome 16. This embryo was therefore classified as mosaic diploid-aneuploid. Additionally, the CGH result for the TE sample biopsied from blastocyst 18-1 was 47,XXY, whereas the FISH result for the corresponding fixed cells (11 cells) showed that three cells were XY, six cells were XY,-20 , and two cells were XY,+20 . Therefore, this embryo was also classified as mosaic diploid–aneuploid.

Table 3-1: FISH and CGH results of blastocysts from 12 patients and origin of aneuploidy

Embryo no.	CGH result	No. cells tested by FISH	FISH result (no. of cells in mosaic embryos)	Agreement FISH and CGH results	Characterization	Origin of error*	Type of aneuploidy event
4-1	46,XX	12	XX	Yes	Normal	-	-
4-2	46,XY	15	XY	Yes	Normal	-	-
4-3	46,XX	18	XX	Yes	Normal	-	-
4-4	46,XX	16	XX	Yes	Normal	-	-
5-1	47,XY,+12	10	XX,+12 (in all cells	Yes	Aneuploid	Meiotic	CG
5-2	48,XY,+15,+16	56	XY (45);XY, +15 (2); XY, +16 (9)	Yes	Mosaic diploid-aneuploid	Post-zygotic	CG
5-3	46,XX	10	XX	Yes	Normal	-	-
5-4	45,XX,-18	15	XX,-18 (all cells)	Yes	Aneuploid	Meiotic	CL
8-1	47,XY,+22	10	XX,+22 (all cells)	Yes	Aneuploid	Meiotic	CG
8-2	47,XY,+4	14	XY,+4 (7); XY,+4,-18 (4); XY,-18 (2); XY (1)	Yes	Mosaic aneuploid	Post-zygotic	CG and CL
8-3	46,XY,-6,+22	16	XY,-6,+22 (9); XY, +22 (7)	Yes	Mosaic aneuploid	Meiotic and post-zygotic	CG and CL
9-1	46,XY	10	XY	Yes	Normal	-	-
9-2	46,XY,-22	13	XY,-22	Yes	Aneuploid	Meiotic	CL
11-1	46,XY,-13,+22	15	XY,-13,+22	Yes	Aneuploid	Meiotic	CG and CL
11-2	45,XY,-15	11	XY,-15 (all cells)	Yes	Aneuploid	Meiotic	CL
11-3	48,XX,+15,+22	17	XX,+15 (9); XX,+15,+22 (8)	Yes	Aneuploid	Meiotic and post-zygotic	CG
12-1	47,XY,+15	10	XY,+15 (all cells)	Yes	Aneuploid	Meiotic	CG

12-2	46,XY	10	XY	Yes	Normal	-	-
13-1	46,YO,+22	16	XY,-16 (10); XY (6)	No	Mosaic diploid-Aneuploid	Post-zygotic	CG and CL
13-2	45,XX,-21	14	XX,-21 (all cells)	Yes	Aneuploid	Meiotic	CL
13-3	47,XY,+16	17	XY,+16 (all cells)	Yes	Aneuploid	Meiotic	CG
13-4	45,XX,-8	24	XX,+15 (10); XX (6); XX,-8 (3), XX,+8 (1); XX,+22 (2); XX,-22 (2)	Yes	Mosaic diploid-aneuploid	Post-zygotic	CG and MND
13-5	46,XY,+6,-16	12	XY (4); X0, -16 (4);X0 (3); XY,+6,-16 (2)	Yes	Mosaic diploid-aneuploid	Post-zygotic	CG and CL
15-1	47,XY,+16	47	XY (34); XY,+16 (7); XY,-16 (2); 5n (3); 6n (1)	Yes	Mosaic diploid-aneuploid	Post-zygotic	MND
15-2	44,XX,-21,-22	39	XX,-21,-22 (30); XX,-22 (5); 4n (4)	Yes	Mosaic aneu-ploid	Meiotic and post-zygotic	CL
17-1	45,XY,-22	14	XY,-22	Yes	Aneuploid	Meiotic	CL
18-1	47,XXY	11	XY,-20 (6), XY (3); XY,+20 (2)	No	Mosaic diploid-aneuploid	Post-zygotic	CG and MND
18-2	45,XY,-18	19	XY,-18 (all cells)	Yes	Aneuploid	Meiotic	CL
19-1	49,XX,+3,+9,+11,-16,+21	31	XX,-3,-9,-16 (12); XX,+11 (9); XX, +21 (4); XX,-21 (4); XX,-11 (3)	Yes	Mosaic aneu-ploid	Post-zygotic	CL and MND
19-2	47,XX,+9,+14,-15	17	XX,+9,+14,-15	Yes	Aneuploid	Meiotic	CG and CL
20-1	44,XY,+7,-18,-20,-21	62	XY,-18 (22); XY,+7,-16 (17); XY ,-21 (8); XY,-20 (8); XY,+7 (7)	Yes	Mosaic aneu-ploid	Post-zygotic	CG and CL
20-2	47,XY,+7,+12,-13	28	XY,+7 (4); XY,+7,+12,+13 (3); XY,+12 (5); XY-16 (6)	Yes	Mosaic aneu-ploid	Post-zygotic	CG, CL and MND

CG: chromosome gain. CL: chromosome loss. MND: mitotic non-dysjunction. *: the origin of type of aneuploidy event can only be estimated based upon the data.

3.2.2 Cytogenetic findings of blastocysts examined by CGH, aCGH and FISH

Twenty of the 52 blastocysts were biopsied twice; the first biopsy was examined with the use of CGH and the second tested via aCGH. As previously, the remainder of each embryo was spread on microscope slides and examined with FISH in two or three rounds (Table 2-3 from above). These blastocysts were donated form nine couples (Table 3-1) with an average maternal age of 33.6 years (age range 24–40 years) [patient 15 had 2 embryos tested with CGH and FISH and only one tested with FISH, CGH and aCGH].

The mean number of cells assessed by FISH for this group of embryos was 50 per blastocyst, while the TE biopsies were estimated to consist of 5–10 cells. Results of the analysed blastocysts are summarized in Table 3-2. FISH and aCGH were successful for all 20 embryos, while CGH failed to give a result for one TE sample (derived from embryo 10-1). The results obtained from the three tests were concordant in all cases with the exception of embryos 3-3 and 15-3, for which CGH and aCGH gave different cytogenetic interpretations. However, the FISH results for these embryos confirmed both CGH and aCGH results, indicating that the embryos were therefore mosaic diploid-aneuploid. As far as embryo 3-3 is concerned, the metaphase CGH result was 47,XY,+16 and aCGH result was 46,XY. FISH showed that 41/93 cells were normal for the tested chromosomes (listed in Table 3-2) and 52/93 cells had a gain of chromosome 16. Additionally, the metaphase CGH result on the TE sample from embryo 15-3 was 47,XX,+20 while the aCGH result on the same embryo was 46,XX. FISH results from the remaining cells of the same embryo showed that 69/90 cells were normal for all of the tested chromosomes (listed in Table 3-2), another 15/90 cells showed a gain of chromosome 13, 4/90 cells had a gain of chromosome 20 and 2/90 cells had a loss of chromosome 20.

Figure 3-1 illustrates the CGH, aCGH and FISH results obtained after the analysis of embryo 16-2, which was classified as uniformly aneuploid by all three methods, carrying an extra copy of chromosome 6 (47,XX,+6).

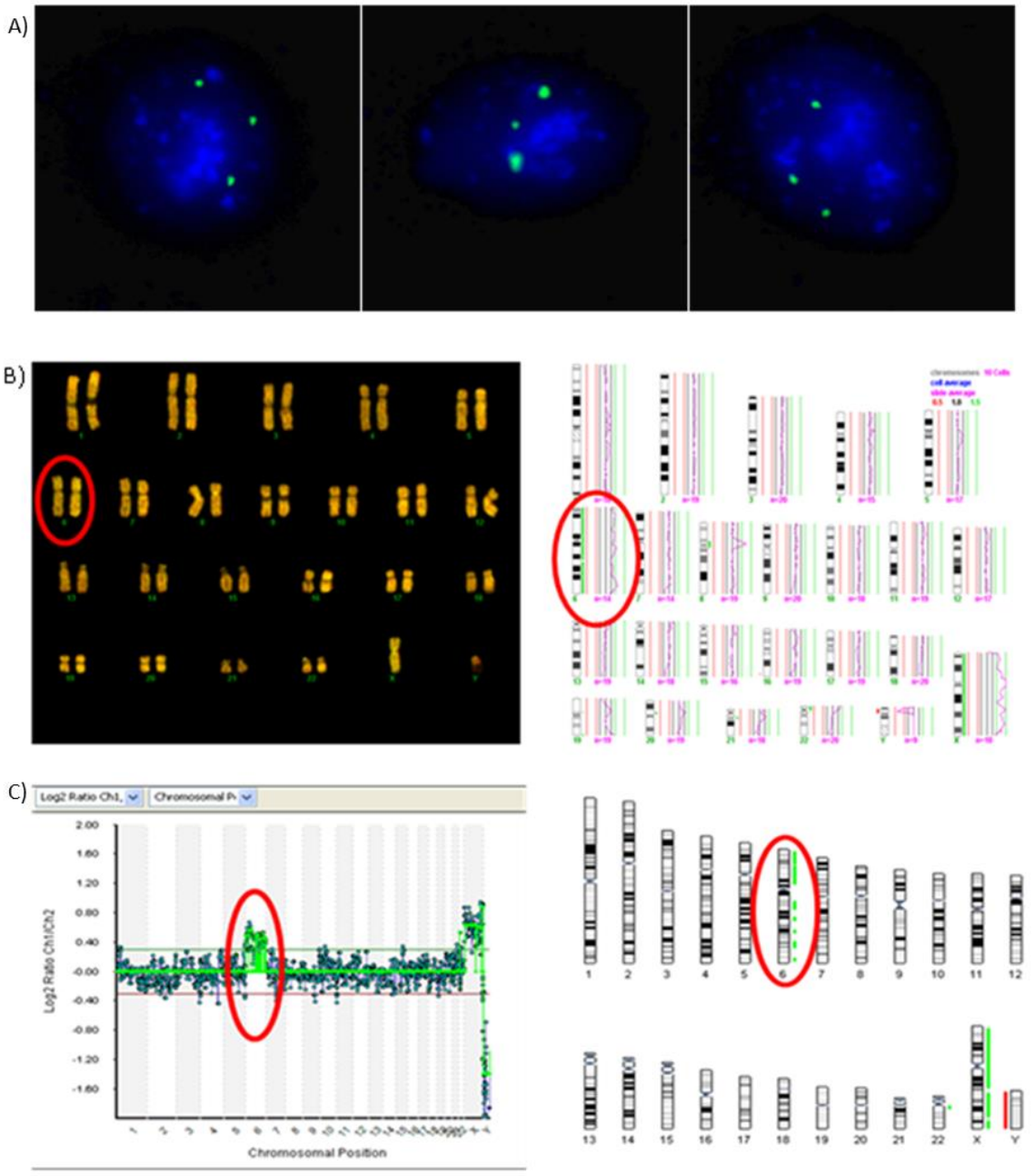
Table 3-2: FISH, CGH and aCGH results of blastocysts from twenty patients and origin of aneuploidy

Embryo no.	CGH result	aCGH result	No. cells tested by FISH	FISH result (no. of cells in mosaic embryos)	Agreement FISH, CGH and aCGH results	Characterization	Origin of error*	Type of aneuploidy event
1-1	46,XY	46,XY	292	XY	Yes	Normal	-	-
2-1	46,XY	46,XY		XY	Yes	Normal	-	-
3-1	46,XY	46,XY	141	XY	Yes	Normal	-	-
3-2	46,XX	46,XX	104	XX	Yes	Normal	-	-
3-3	47,XY,+16	46,XY	93	XY,+16 (52); XY (41)	FISH and CGH/ FISH and aCGH	Mosiac diploid-aneuploid	Post-zygotic	CG
3-4	46,XY	46,XY	155	XY	Yes	Normal	-	-
6-1	46,XX	46,XX		XX	Yes	Normal	-	-
6-2	46,XX	46,XX		XX	Yes	Normal	-	-
7-1	46,XX	46,XX	99	XX	Yes	Normal	-	-
7-2	46,XX	46,XX	123	XX	Yes	Normal	-	-
7-3	46,XY	46,XY	427	XY	Yes	Normal	-	-
7-4	46,XY	46,XY	66	XY	Yes	Normal	-	-
10-1	No result	46,XX	11	XX	FISH and aCGH	Normal	-	-
10-2	46,XY	46,XY	150	XY	Yes	Normal	-	-
14-1	46,XY	46,XY	74	XY	Yes	Normal	-	-
14-2	46,XY	46,XY	56	XY	Yes	Normal	-	-
15-3	47,XX,+20	46,XX	90	XX (69); XX,+13 (15); XX,+20 (4); XX,-20 (2)	FISH and CGH/ FISH and aCGH	Mosiac diploid-aneuploid	Post-zygotic	CG and MND
16-1	47,XXY	46,XX	28	Chaotic	Yes	Chaotic	Post-zygotic	Aneuploidy event not defined
16-2	47,XX,+6	47,XX,+6	53	XX,+6	Yes	Aneuploid	Meiotic	CG
16-3	47,XX,+15	48,XX,+15,+22	21	XX,+22 (11), XX,	Yes	Mosiac diploid-aneuploid	Post-zygotic	CG and MND

				+15 (7), XX (3), XX, -15				

CG: chromosome gain. CL: chromosome loss. MND: mitotic non-dysjunction. *: the origin of type of aneuploidy event can only be estimated based upon the data.

Figure 3-1: A concordant FISH, metaphase and array CGH results for embryo 16-2



A) FISH result on three nuclei from embryo 16-2 showing three signals for centromeric probe for chromosome 6. B) Metaphase CGH result showing a female embryo with an extra copy of chromosome 6. C) Array CGH results from the same embryo with karyotype 47,XX,+6. (As shown also in Fragouli et al. 2011c).

3.2.3 Summary of results obtained during the comprehensive examination of blastocysts using FISH, CGH and aCGH

All three techniques performed well; FISH yielded results for all embryos, CGH failed for one of the fifty-two tested embryos and aCGH gave results for all tested embryos (success rate was 100%, 98% and 100%, respectively). Control lymphocyte slides were examined to allow the efficiency of each of the probe mixtures to be determined. Probe panel efficiencies ranged between 95 and 98%.

It was evident from the obtained results that twenty-two embryos (42.3%) were uniformly normal (embryos were considered uniformly euploid when they presented normal results for metaphase CGH, aCGH and ≥ 95% of the cells were diploid for the tested chromosomes with FISH), twenty-one (40.4%) had abnormalities present in all their cells (aneuploid or mosaic aneuploid) and nine (17.3%) embryos were characterised as mosaic diploid-aneuploid (consisting of normal and abnormal cell lines).

Almost all chromosomes were seen to be affected by aneuploidies originating either during meiosis and/or post-zygotically (Table 3-3). Chromosomal abnormalities of presumed meiotic origin (present in every cell) were seen in 16 blastocysts (30.7%). In thirteen (81%) of blastocysts with such an abnormality no other aneuploid chromosomes were detected, while the other three embryos (19%) had additional post-zygotic errors. Eleven of the 13 (85%) embryos with meiotic aneuploidies only were abnormal for a single chromosome, while embryos 11-1 and 19-2 (Table 3-5) were affected by two and three meiotic abnormalities, respectively. A total of 19 chromosome anomalies occurred during meiosis in the investigated group of blastocysts. Ten (53%) of these were gains of one or more chromosomes, while the remaining 9 (47%) were monosomies.

In addition, 40 chromosome abnormalities of likely post-zygotic origin were detected; of these, 16 events involved chromosome gain (CG), 13 events were associated with chromosome

loss (CL) and 11 events were a consequence of mitotic non-disjunction (MND), reciprocal losses and gains of the same chromosome seen in different cells of the embryo).

This data confirms that mosaicism remains a common phenomenon even during this final stage of development immediately prior to implantation (i.e. the blastocyst stage). Specifically, 17/52 analysed embryos (32.7%) were characterized as being mosaic. Eight (47%) of these blastocysts were classified as being mosaic aneuploid, carrying different types of abnormalities in all of their cells, while the remaining nine (53%) were characterized as mosaic diploid-aneuploid. Three of these embryos (embryos 5-1, 15-1 and 15-3) had a normal chromosome complement in the majority of their cells (>70%).

Fourteen of the mosaic embryos had only aneuploidies of post-zygotic origin, and the other three had a combination of meiotic and post-zygotic errors. It is also very interesting that only one of the 52 blastocysts (2%) was found to have a chaotic chromosome complement, composed of multiple different aneuploid cell lines.

Table 3-3: Number and type and origin of errors affecting each chromosome.

Chromosome	Meiotic origin		Post-zygotic origin			Total
	Gain	Loss	Gain	Loss	MND	
1	-	-	-	-	-	0
2	-	-	-	-	-	0
3	-	-	-	-	1	1
4	-	-	1	-	-	1
5	-	-	-	-	-	0
6	1	-	1	1	-	3
7	-	-	2	-	-	2
8	-	-	-	-	1	1
9	1	-	-	-	1	2
10	-	-	-	-	-	0
11	-	-	-	-	1	1
12	1	-	1	-	-	2
13	-	1	1	-	1	3
14	1	-	-	-	-	1
15	2	2	2	-	1	7
16	1	-	2	5	1	9
17	-	-	-	-	-	0
18	-	2	-	2	-	4
19	-	-	-	-	-	0
20	-	-	-	1	2	3
21	-	1	-	2	1	4
22	3	3	3	-	1	10
XY	-	-	2	2	-	4
Total	Meiotic gain	Meiotic loss	Post-zygotic gain	Post-zygotic loss	Post-zygotic MND	Total abnor-malities
	10	9	15	13	11	58

In order to examine the effect of maternal age on the chromosomes of human blastocysts, we divided the participating patients into a younger and an older group. The younger group included 8 patients and had an average maternal age of 30.6 years (24-36 years). Twenty three blastocysts were analysed from these patients and 7 were chromosomally abnormal (two of them were characterized as mosaic diploid-aneuploid) leading to an aneuploidy rate of 30.4%. However, for the second older group, which included 12 patients with average maternal age of 39.8 years (age range 37-42 years), 29 blastocysts were tested and 22 were chromosomally abnormal, leading to an aneuploidy rate of 75.9% (seven of them were characterized as mosaic diploid-aneuploid). These results are indicative of a significant (P = 0.002, Fisher's exact test) increase to the chromosome abnormalities taking place in blastocysts associated with advancing female age.

The total number of errors observed in the younger patient group were 10, 4 of them were of meiotic origin (40%) and 6 (60%) were of post-zygotic origin. For the older group, 48 errors were observed and 15 (31.25%) were of presumed meiotic origin and 33 (68.75%) were of post-zygotic origin. The number of errors was too small to allow a meaningful statistical assessment.

3.2.4 The use of CGH/aCGH for the analysis of biopsied TE samples

Evaluation and comparison of the cytogenetic results obtained with the use of all three methods suggested that both CGH and aCGH are capable of reliably diagnosing aneuploidy in the vast majority of cases, although there remains a chance that abnormalities present in a small minority of cells might not be detected. Aneuploidies were correctly identified in all embryos in which at least 60% of the cells were affected by the abnormality (as observed from embryo 3-3 where 55% of its cells had an aneuploidy that was not detected by metaphase CGH). However, it is important to note that the loss or gain of an entire set of chromosomes (i.e. ploidy change) cannot be detected using CGH or aCGH. FISH analysis of the 52 blastocysts revealed the presence

of variable numbers of polyploid cells in 12 embryos. In the vast majority (10/12) of these embryos the ploidy change scored was tetraploidy. The presence of tetraploid cells is considered a natural phenomenon during the final stages of embryonic development, possibly related to the initiation of trophoblast differentiation (Bielanska et al., 2002).

3.3 Summary of findings

This study has shown that metaphase CGH and aCGH provide accurate tools to investigate the chromosome constitution of embryos at the blastocyst stage. Moreover, 42% of blastocysts were found to be chromosomally normal, which is a considerably higher proportion of normal embryos in comparison to the cleavage stage. While the overall mosaicism rate for all embryos was around 30%, only 17% of blastocysts were a mixture of normal and abnormal cells (i.e. mosaic diploid-aneuploid). Furthermore, the proportion of mosaic diploid-aneuploid blastocysts having more then half of their cells normal was very small. Therefore, the risk of misdiagnosis is very low at the blastocyst stage using comprehensive screening techniques such as metaphase CGH or aCGH.

4. The effect of aneuploidy on embryo morphology and development to the cleavage and blastocyst stage

4.1 Aims

A large number of investigations examining the chromosome complement of cleavage and blastocyst stage embryos have clearly shown that numerical abnormalities (aneuploidy) are very common. Such chromosomal abnormalities are believed to be an important cause of implantation failure and miscarriage (Delhanty et al., 1993; Munné et al., 1993; Iwarsson et al., 1999; Wells and Delhanty, 2000; Wilton et al., 2003; Fragouli et al., 2008; 2010; 2011). Very few studies, however, have investigated the possibility of associations between aneuploidy and embryo morphology at different developmental stages.

The aim during this part of the project was to test test the hypothesis that aneuploidy affects the embryo morphology and development. For this purpose, the copy number of all 24 chromosomes was evaluated during progression from the cleavage to blastocyst stage. Particular attention was paid to the morphology of embryos carrying chromosome errors capable of implanting and forming a clinical pregnancy (and in some cases a live birth- i.e. trisomies 13, 16, 18, 21, 22, and aneuploidy affecting the sex chromosomes). The morphological appearance of embryos with these aneuploidies was carefully compared to those with a euploid chromosome constitution.

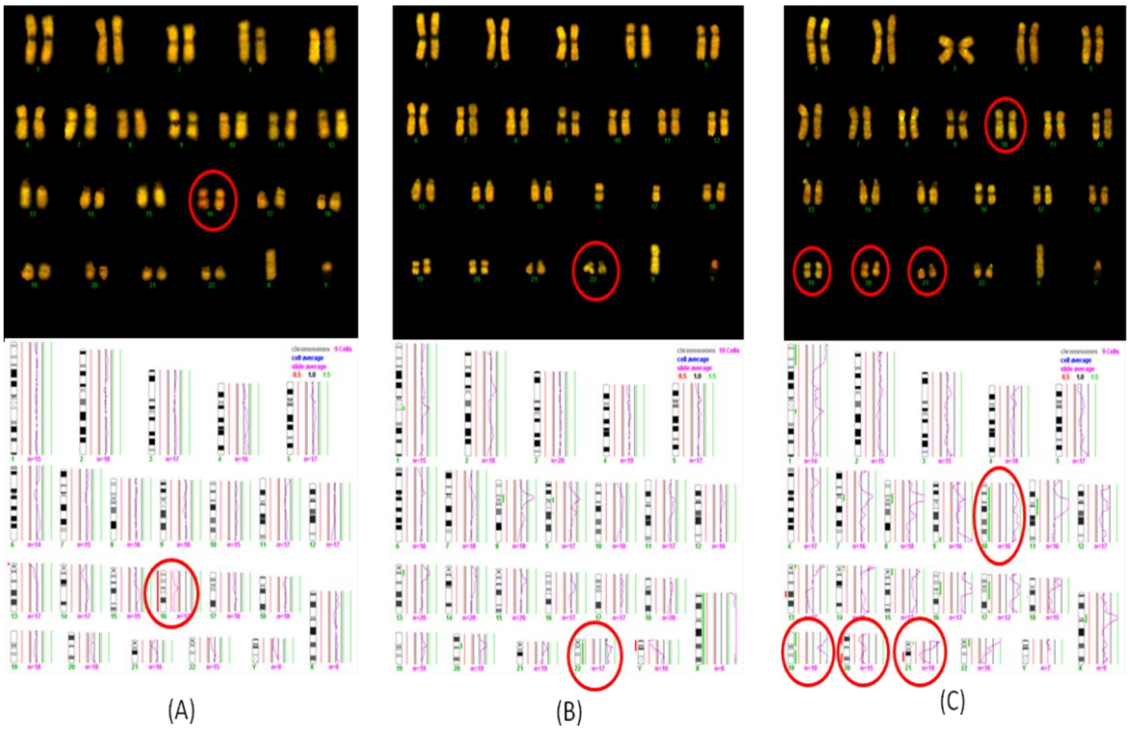
4.2 Results

4.2.1 Cleavage stage

4.2.1.1 The incidence of chromosome abnormalities at the cleavage stage

Three hundred and eighty seven (89%) of the blastomeres biopsied from cleavage stage embryos were successfully analysed during the current study. Of these samples, 64 (16.5%) were found to be chromosomally normal and 323 (83.5%) were abnormal. Upon further investigation, it was seen that 97 (25.1%) of all embryos carried a single aneuploidy, 66 (17%) carried two distinct aneuploidies, and 160 (41.4%) carried three or more aneuploidies. Patient and embryo details are presented in Table B-1 in Appendix B. Figure 4-1 shows some examples of the metaphase CGH profile of abnormal embryos and Figure 4-2 shows some aCGH profile of abnormal embryos.

Figure 4-1: Examples of metaphase CGH result profiles



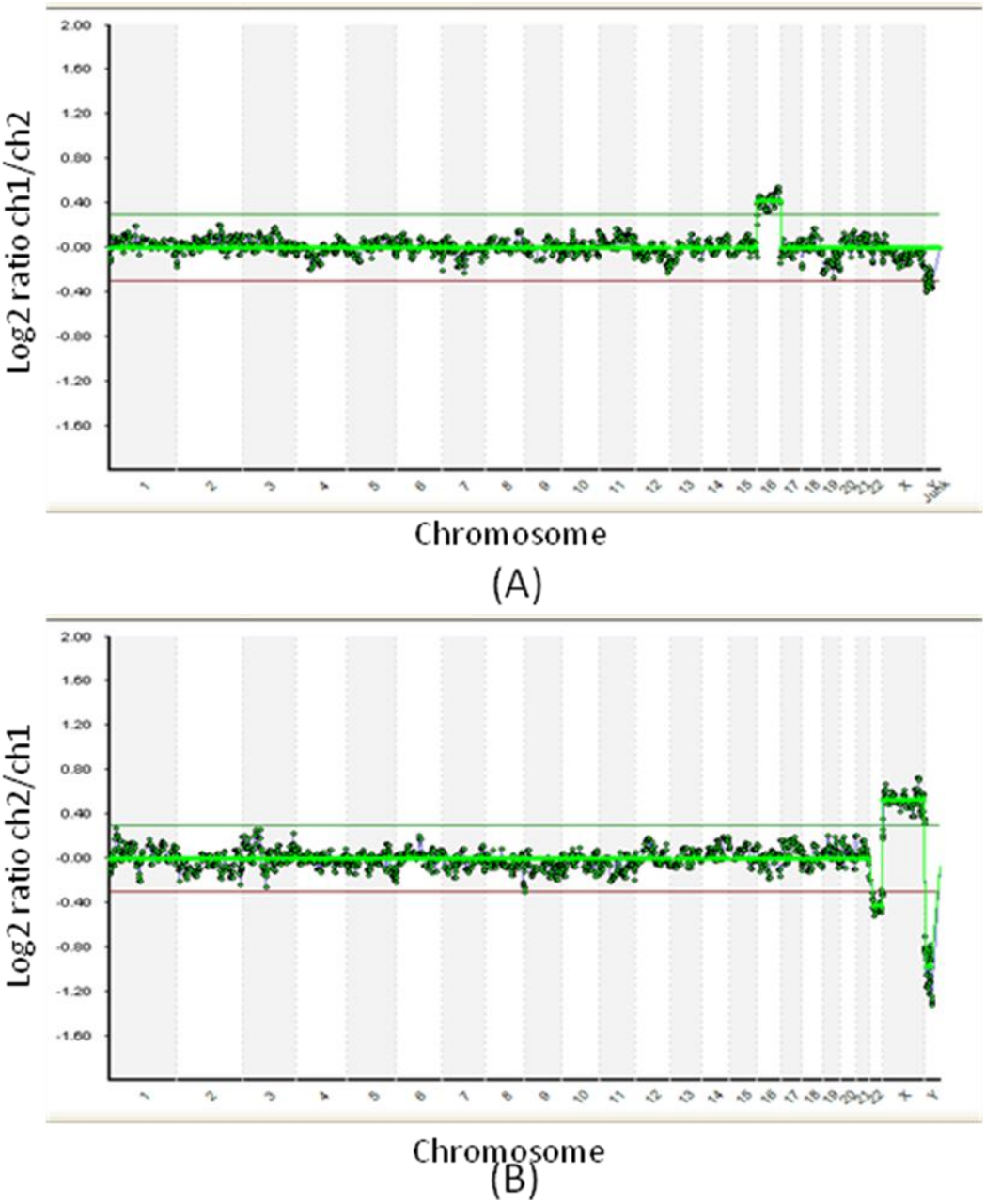
(A) TE biopsy from embryo 8, patient 12 (45,XY,-16)*

(B) TE biopsy from embryo 2, patient 19 (47,XX,+22)*

(C) TE biopsy from embryo 10, patient 111 (46,XX,+10,+19,-20,-21)*

* All images are courtesy of *Samer Alfarawati*

Figure 4-2: Examples of aCGH result profiles



(A) Blastomere biopsy from embryo 1, patient 19 (47,XY,+16)

(B) Blastomere biopsy from embryo 8, patient 5 (45,XX,-22)

A total of 1,293 chromosome errors were scored in the abnormal blastomeres biopsied from cleavage-stage embryos. Of these, 560 were trisomies and 649 were monosomies of whole chromosomes, while the remaining 84 were partial (segmental) aneuploidies, presumably generated following chromosome breakage. Most (55 out of 83) of these partial chromosome abnormalities affected the larger chromosomes 1-12 (Figure 4-3). A breakdown of abnormalities scored per chromosome is illustrated in Table 4-1. The top five chromosomes in terms of aneuploidy risk were 22, 15 and 16 (equal), X/Y, 19 and 21 in order of frequency.

Figure 4-3: The frequency of chromosome abnormalities in cleavage stage embryos

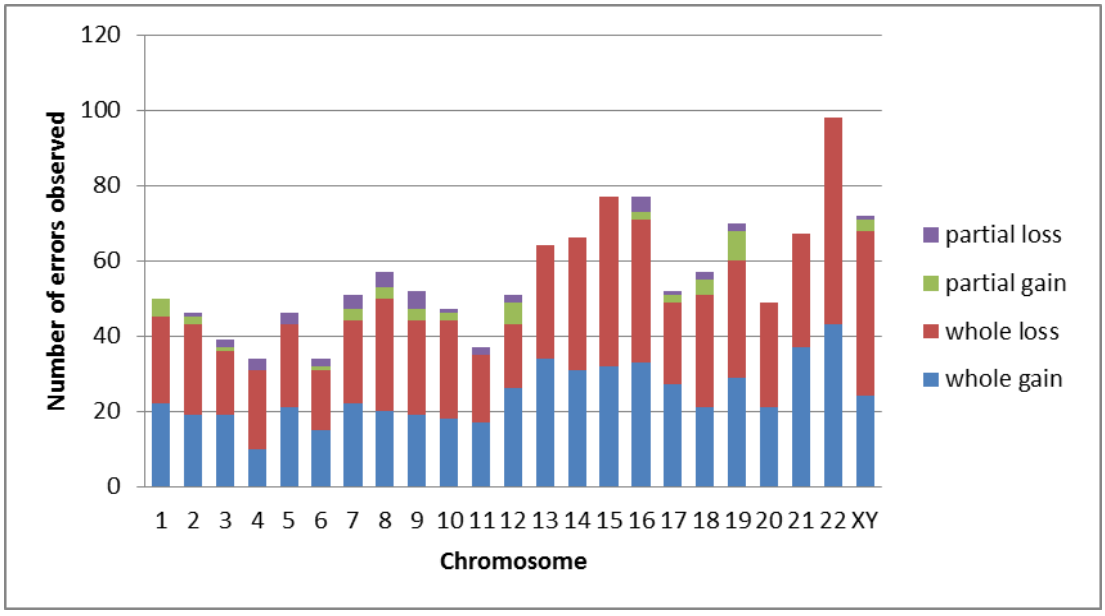


Table 4-1: Gains and losses of chromosomes in cleavage stage embryos

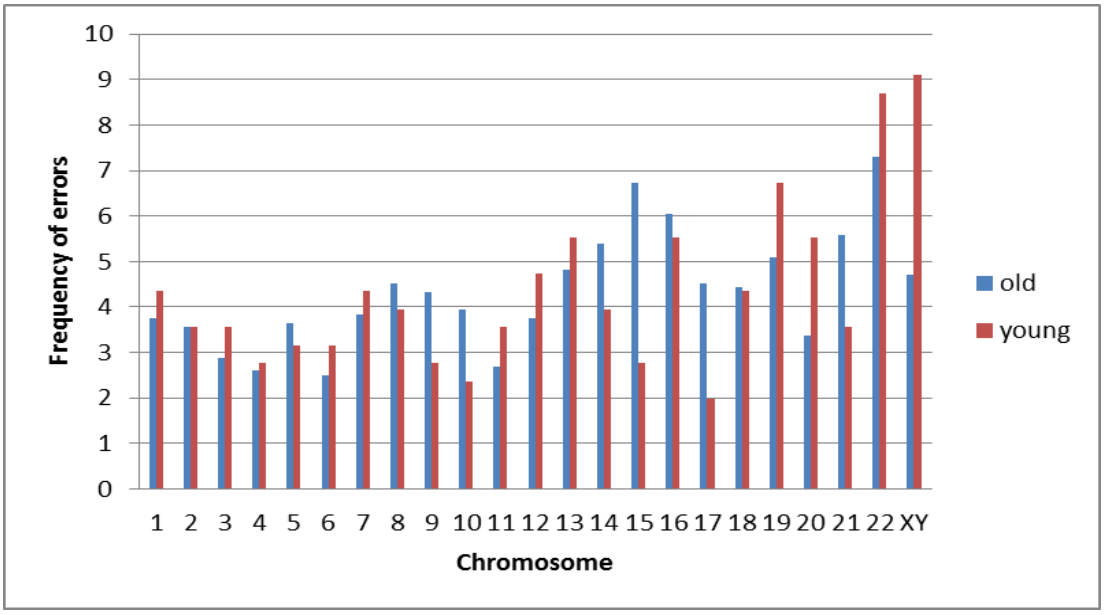
Chromosome	whole gain	whole loss	partial gain	partial loss	Total
1	22	23	5	0	50 (3.9%)
2	19	24	2	1	46 (3.6%)
3	19	17	1	2	39 (3%)
4	10	21	0	3	34 (2.6%)
5	21	22	0	3	46 (3.6%)
6	15	16	1	2	34 (2.6%)
7	22	22	3	4	51 (3.9%)
8	20	30	3	4	57 (4.4%)
9	19	25	3	5	52 (4%)
10	18	26	2	1	47 (3.6%)
11	17	18	0	2	37 (2.9%)
12	26	17	6	2	51 (3.9%)
13	34	30	0	0	64 (4.9%)
14	31	35	0	0	66 (5.1%)
15	32	45	0	0	77 (6%)
16	33	38	2	4	77 (6%)
17	27	22	2	1	52 (4%)
18	21	30	4	2	57 (4.4%)
19	29	31	8	2	70 (5.4%)
20	21	28	0	0	49 (3.8%)
21	37	30	0	0	67 (5.2%)
22	43	55	0	0	98 (7.6%)
XY	24	44	3	1	72 (5.6%)
Total	560	649	45	39	1293

4.2.1.2 The effect of age on embryonic chromosomal status at the cleavage stage

The embryos were also assessed in relation to female age. Specifically, the samples were divided into two sub-groups; the first composed of embryos generated by 14 younger women (average age: 33.4 years, range 27-36 years), the second consisting of embryos from 53 older women (average age: 40.8 years, range: 37-45 years). Of the 86 embryos analysed for the younger group, 24 (28%) were seen to be euploid, and the remaining 62 (72%) were aneuploid. For the older patient group, 301 embryos were examined, 39 (13%) of which were normal, and 262 (87%) were aneuploid. The difference seen in aneuploidy rates between the younger and older groups of women was highly statistically significant ($P<0.002$, Fisher's exact test).

A comparison of the chromosomes most frequently scored as aneuploid in the embryos of younger and older patients also took place. Interestingly, different chromosomes were affected by malsegregation errors for the two different age groups. Hence, chromosomes X/Y, 22, 19, 20 and 16 were most often scored as abnormal in embryos generated by younger women, whereas chromosomes 22, 15, 16, 21 and 14 were more frequently affected in embryos of older women. These results are shown in Figure 4-4.

Figure 4-4: The frequency of chromosome abnormalities in younger and older patient groups showing differences in the frequency of aneuploidy between the two groups



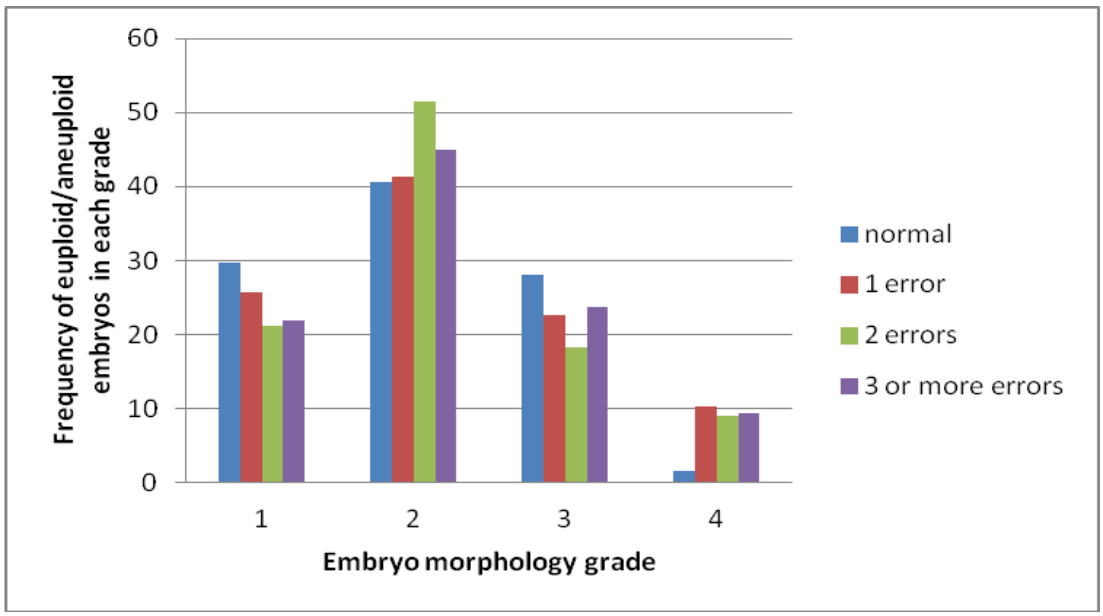
4.2.1.3 The effect of chromosome abnormalities on cleavage stage embryo morphology

All analysed embryos were classified according to morphological criteria described in Table 1 (Grade 1-4). Aneuploid embryos were also grouped according to the number of errors they were identified to be carrying, i.e. one, two, or three or more chromosome abnormalities. These results are shown in Table 4-2. Figure 4-5 shows the proportion of normal and abnormal embryos with different number of errors for all morphological grades.

Table 4-2: Cleavage stage embryo quality and chromosomal status

Embryo Grade	Normal embryos	Abnormal embryos			
		1 error	2 errors	3 or more errors	Total abnormal
1	19 (29.7%)	25 (25.8%)	14 (21.2%)	35 (21.9%)	74 (22.9%)
2	26 (40.6%)	40 (41.2%)	34 (51.5%)	72 (45%)	146 (45.2%)
3	18 (28.1%)	22 (22.7%)	12 (18.2%)	38 (23.75%)	72 (22.3%)
4	1 (1.6%)	10 (10.3%)	6 (9.1%)	15 (9.4%)	31 (9.6%)
Total	64 (100%)	97 (100%)	66 (100%)	160 (100%)	323 (100%)

Figure 4-5: The distribution of embryos of different chromosomal status in each morphological grade. No differences observed except for embryos with worst quality (grade 4)



Approximately 30% of chromosomally normal embryos were classified as good quality (grade 1), but 26% of abnormal embryos carrying a single chromosome error, 21% of those with 2 errors, and 22% of those carrying 3 or more errors (complex aneuploid), were also classified as grade 1. It was therefore evident that on day-3, amongst the embryos of best morphological quality, there was no significant difference in the incidence or extent of aneuploidy (Fisher's exact test, P = 0.23).

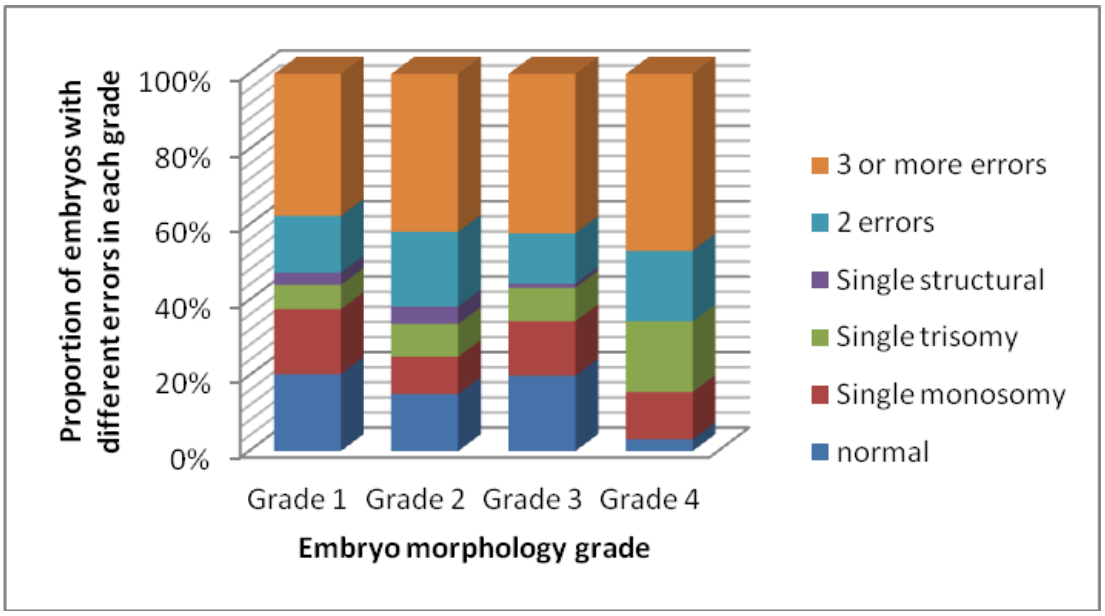
Conversely, only 1.6% of euploid embryos were of the poorest morphological grade (grade 4), whilst 10.3% of those with a single chromosome error, 9.1% of embryos with 2 errors, and 9.4% of those with complex aneuploidy were grade 4. It was therefore significantly more likely for a chromosomally abnormal embryo to be of the poorest morphological grade. This was true regardless of whether the abnormality involved one chromosome or multiple chromosomes (Fisher's exact test, P = 0.04 and P = 0.05, respectively).

It should be noted that at the cleavage stage complex aneuploidy was present regardless of embryonic grade and no significant difference was observed between the frequency of abnormal embryos with complex error of grade 1 (35/93) and those of grade 4 (15/32) (Fisher's exact test, P = 0.4). Interestingly, monosomies (16/93) were more frequently observed than trisomies (6/93) in the aneuploid grade 1 embryos (2.7:1) and a significant difference was observed when their frequency was compared to the total number of embryos of grade 1 (Fisher's exact test, P = 0.04), whereas the opposite was observed for grade 4 aneuploid embryos (1:1.5) but no significant difference was observed (Fisher's exact test, P = 0.73). These findings are illustrated in Table 4-3 and Figure 4-6 below.

Table 4-3: Normal and abnormal cleavage-stage embryos with different morphological grades

	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Normal	19 (20.4%)	26 (15.1%)	18 (20%)	1 (3.1%)
Single monosomy	16 (17.2%)	17 (9.9%)	13 (14.4%)	4 (12.5%)
Single trisomy	6 (6.5%)	15 (8.7%)	8 (8.9%)	6 (18.75%)
Single structural	3 (3.2%)	8 (4.6%)	1 (1.1%)	0 (0%)
2 errors	14 (15.1%)	34 (19.8%)	12 (13.3%)	6 (18.75%)
3 or more errors	35 (37.6%)	72 (41.9%)	38 (42.2%)	15 (46.9%)
Total	93 (100%)	172 (100%)	90 (100%)	32 (100%)

Figure 4-6: Cleavage-stage morphological grading and the proportion of normal and abnormal embryos. Significant differences in the frequency of euploid embryos only observed in grade 4



4.2.1.4 The correlation between cell number at the cleavage stage and chromosomal status

Embryos at the cleavage stage were divided into 3 categories according to the number of cells on day-3: those with < 6 cells; between 6-9 cells; embryos with 10 or more cells. The majority of embryos had 6-9 cells, as typically expected on day 3 of embryonic development. However, no statistical significance was observed between normal and abnormal embryos with different number of cells. These results are shown in Table 4-4.

Table 4-4: Cell number and embryonic karyotype. No differences observed between faster or slower embryos.

Chromosomal status	< 6 cells	6-9 cells	10 or more cells
Normal	5 (14.3%)	51 (16%)	7 (22%)
Abnormal	30 (85.7%)	267 (84%)	25 (78%)
Total	35 (100%)	318 (100%)	32 (100%)

4.2.1.5 Morphology at the cleavage stage and embryo gender

A total of 327 cleavage stage embryos had a normal sex chromosome complement. Of these, 136 (41.6%) were male and 191 (58.4%) were female, which is a significant deviation of the expected 1:1 ratio (Fisher's exact test, P = 0.03). On day-3 approximately 10% of female embryos consisted of 10 or more cells, but less than 6% of male embryos were at the same stage. However, given the sample size, this difference was not statistically significant (Fisher's exact test, P = 0.2). No significant difference was observed between the sex of embryos and their euploidy/aneuploidy status, i.e. the risk of aneuploidy was identical for the two sexes. Table 4-5 describes this set of data.

Table 4-5: Normal and abnormal male and female embryos and corresponding cell number at the cleavage stage showing no differences in aneuploidy rate between genders

Number of cells	Female embryos		Male embryos	
	Normal	Abnormal	Normal	Abnormal
< 6 CELLS	3 (1.6%)	14 (7.3%)	2 (1.5%)	13 (9.6%)
6-9 CELLS	25 (13.1%)	130 (68.1%)	26 (19.1%)	87 (64%)
≥ 10 CELLS	6 (3.1%)	13 (6.8%)	1 (0.7%)	7 (5.1%)
Total	34 (17.8%)	157 (82.2%)	29 (21.3%)	107 (78.7%)
	191 (100%)		136 (100%)	

4.2.1.6 The morphology of cleavage stage embryos with aneuploidies compatible with implantation and/or live birth

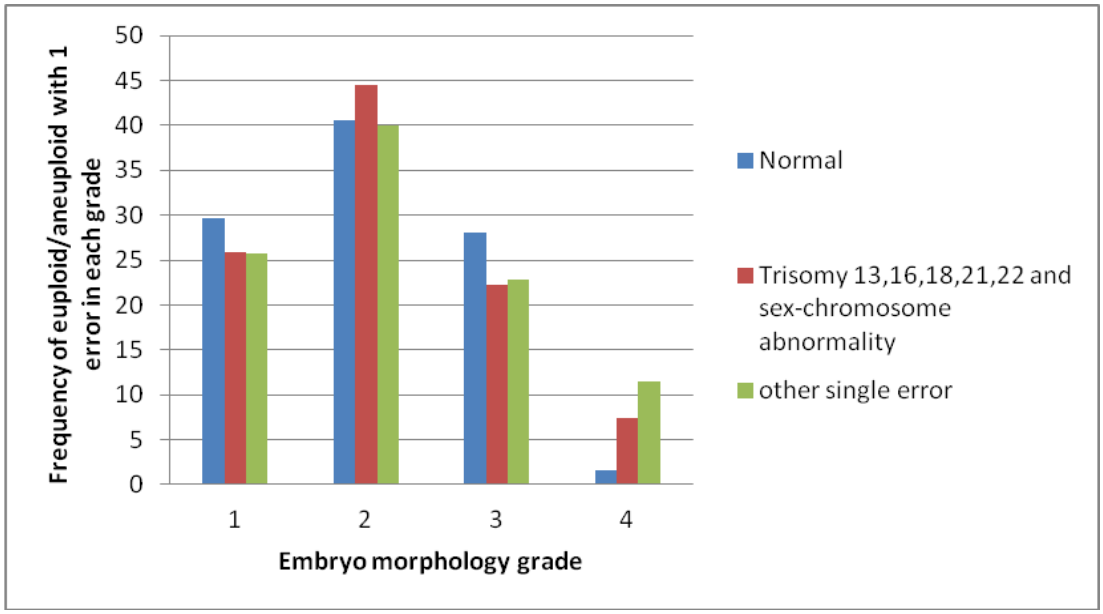
The morphology of aneuploid embryos carrying chromosome errors potentially capable of implanting and forming a clinical pregnancy (i.e. trisomies 13, 16, 18, 21 and 22, and abnormalities of the sex chromosomes) was also evaluated. Specifically, comparison took place between three groups of embryos: those with the aneuploidies listed above (potentially compatible with implantation and pregnancy); embryos that were euploid; those carrying single trisomies affecting other chromosomes or single monosomies (not compatible with initiation of a pregnancy).

Statistical analysis of the data obtained suggested that at the cleavage stage, embryos with these different types of chromosomal constitution have no significant differences between their morphological appearances (grade 1, 2 and 3). However, there was a significant difference between the number of normal and aneuploid embryos with single aneuploidies incompatible with pregnancy with grade 4 morphology (Fisher's exact test, P = 0.035). Table 4-6 and Figure 4-7 demonstrate these results.

Table 4-6: Embryonic morphology and viable aneuploidies**.** No differences found between embryos at all grades and different karyotypes

Embryo grade	Normal embryos	Abnormal embryos (trisomy 13, 16, 18, 21, 22 and sex-chromosome aneuploidies)	Abnormal embryos (1 error excluding column 3)
1	19 (29.7%)	7 (26%)	18 (25.7%)
2	26 (40.6%)	12 (44.4%)	28 (40%)
3	18 (28.1%)	6 (22.2%)	16 (22.9%)
4	1 (1.6%)	2 (7.4%)	8 (11.4%)
Total	64 (100%)	27 (100%)	70 (100%)

Figure 4-7: Embryo morphology, normality and viable and non-viable aneuploidy. No differences found between embryos at different grades



4.2.2 Blastocyst stage

4.2.2.1 The incidence of chromosome abnormalities at the blastocyst stage

Of the blastocysts tested during this part of the project, 868 (93%) yielded a CGH (metaphase or microarray) result. A total of 378 (43.5%) were found to be chromosomally normal, 288 (33.2%) carried a single aneuploidy, 122 (14%) had 2 abnormalities (14%) and 80 (9.3%) were affected by 3 or more errors. Patient and embryo details are presented in Table B-2 in Appendix B.

A total of 861 chromosome abnormalities were scored in the abnormal TE samples, 380 (44%) of which were trisomies, 412 (48%) were monosomies, and 69 (8%) involved partial losses or gains of chromosomal material. The frequency of aneuploidy affecting different chromosomes can be seen in Figure 4-8 and a Table 4-7. The five chromosomes most frequently abnormal at the blastocyst stage were 22, 16, 15, X/Y, and 21.

Figure 4-8: The frequency of chromosome abnormalities at the blastocyst stage

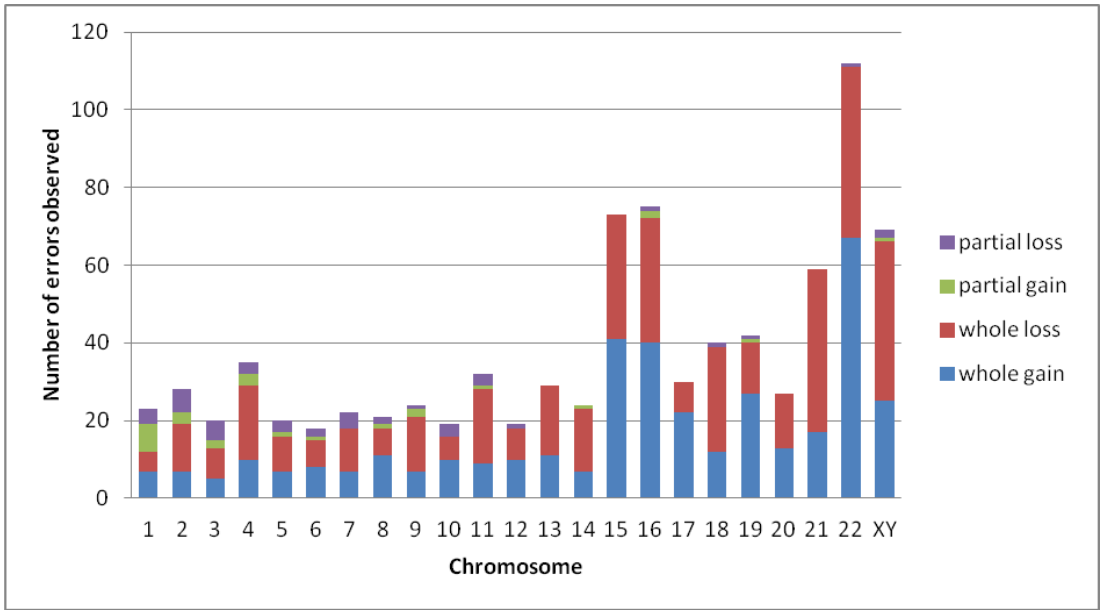


Table 4-7: Gains and losses of chromosomes in embryos at the blastocyst stage

Chromosomes	Whole gain	Whole loss	Partial gain	Partial loss	Total
1	7	5	7	4	23 (2.7%)
2	7	12	3	6	28 (3.3%)
3	5	8	2	5	20 (2.3%)
4	10	19	3	3	35 (4.1%)
5	7	9	1	3	20 (2.3%)
6	8	7	1	2	18 (2.1%)
7	7	11	0	4	22 (2.6%)
8	11	7	1	2	21 (2.4%)
9	7	14	2	1	24 (2.8%)
10	10	6	0	3	19 (2.2%)
11	9	19	1	3	32 (3.7%)
12	10	8	0	1	19 (2.2%)
13	11	18	0	0	29 (3.4%)
14	7	16	1	0	24 (2.8%)
15	41	32	0	0	73 (8.5%)
16	40	32	2	1	75 (8.7%)
17	22	8	0	0	30 (3.5%)
18	12	27	0	1	40 (4.6%)
19	27	13	1	1	42 (4.9%)
20	13	14	0	0	27 (3.1%)
21	17	42	0	0	59 (6.9%)
22	67	44	0	1	112 (13%)
XY	25	41	1	2	69 (8%)
Total	380	412	26	43	861

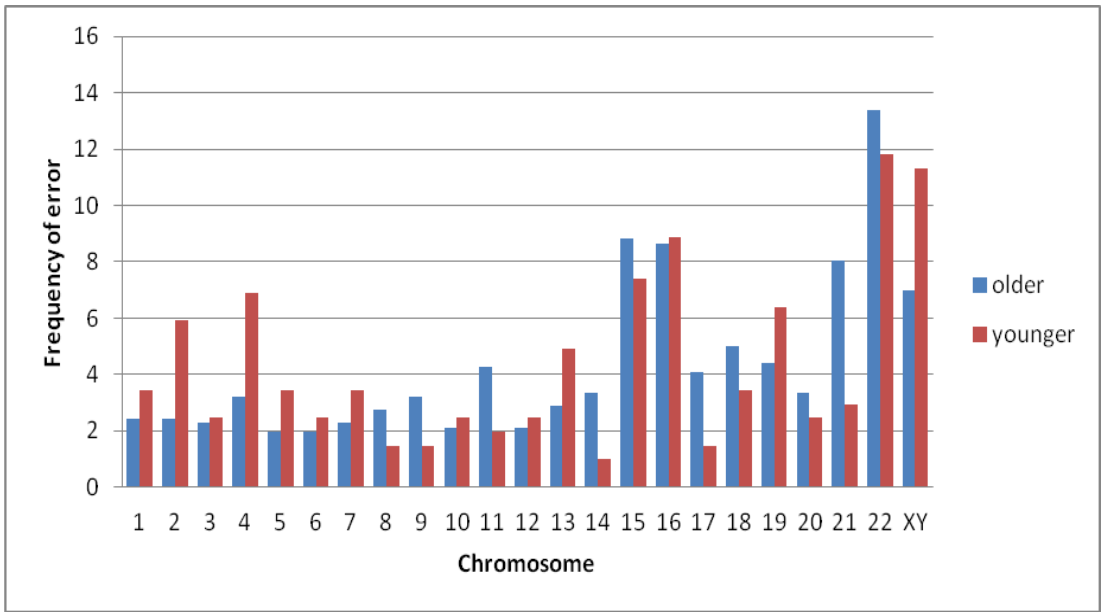
4.2.2.2 The effect of age on embryonic chromosomal status at the blastocyst stage

Similar to the analysis performed at the cleavage stage, blastocyst chromosome constitution was evaluated in relation to female age. This analysis included a total of 290 embryos generated by 44 'younger' women (average age: 33.5 years, range: 29-36 years). Of these, 157 (54%) were euploid, whereas aneuploidy was scored in the remaining 133 (46%).

578 blastocysts were generated by 118 reproductively older women (average age: 40.4 years, range: 37-47 years). Fewer blastocysts were euploid (220, 38%), the majority being chromosomally abnormal (358, 62%). As with cleavage stage embryos, the difference seen in aneuploidy rates between blastocysts from younger and older women was highly statistically significant ($P<0.0001$).

When the frequency of aneuploidy for individual chromosomes was compared between embryos generated by younger and older women, some differences were observed. Chromosomes 22, X/Y, 16, 15 and 4 were most often scored as abnormal in embryos generated from younger women, whereas chromosomes 22, 15, 16, 21 and X/Y were most frequently affected in embryos generated from older women. Figure 4-9 illustrates the differences seen between the two age subgroups.

Figure 4-9: The frequency of chromosome abnormalities in blastocysts generated by younger and older women showing some differences between both groups (X/Y, 21, 14 and 17)



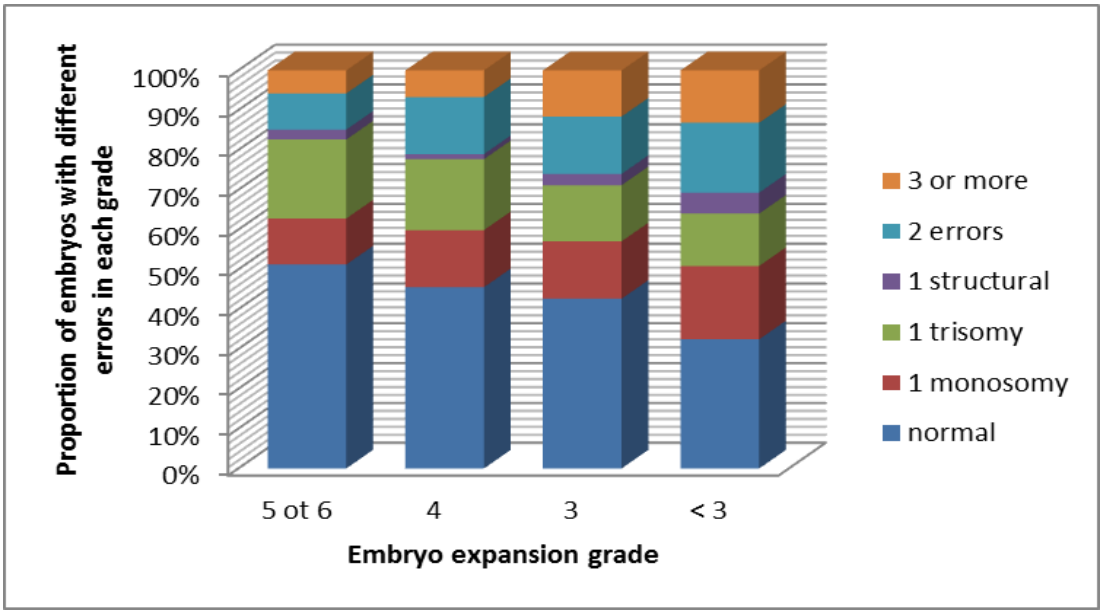
4.2.2.3 The effect of chromosome abnormalities on blastocyst stage morphology

Analysis of the chromosome constitution of embryos which had reached the final stage of preimplantation development, showed that 62/121 (52.1%) of the best quality blastocysts (grades 5-6) were chromosomally normal, with the euploidy rate decreasing as the embryo quality became worse. Specifically, only 32% (37/114) of the poorest quality blastocysts (grades 1-2) were characterised as euploid by aCGH. The opposite was seen for aneuploid blastocysts. It was evident that euploid embryos tend to show a faster progression to the most advanced blastocyst stages when compared to chromosomally abnormal embryos (Fisher's exact test, P = 0.07). Hence, the proportion of abnormal embryos which on day-5 were classified as early blastocysts was significantly higher than the proportion of euploid embryos (Fisher's exact test, P = 0.008). These results are illustrated in Table 4-8 and Figure 4-10.

Table 4-8: Euploid and aneuploid blastocysts and their morphology. A decrease in euploidy rate was observed with decreased embryo quality.

	Embryo expansion grade			
	5 or 6	**4**	**3**	**< 3**
Normal	62 (51.2%)	143 (45.5%)	136 (42.7%)	37 (32.4%)
Single monosomy	14 (11.6%)	45 (14.3%)	46 (14.4%)	21 (18.4%)
Single trisomy	24 (19.8%)	56 (17.8%)	45 (14.1%)	15 (13.2%)
Single structural	3 (2.5%)	4 (1.3%)	3 (2.8%)	6 (5.3%)
2 errors	11 (9.1%)	45 (14.3%)	46 (14.4%)	20 (17.5%)
3 or more errors	7 (5.8%)	21 (6.7%)	37 (11.6%)	15 (13.2%)
Total	121 (100%)	314 (100%)	319 (100%)	114 (100%)

Figure 4-10: Categories of chromosomal constitution grouped according to blastocyst morphology showing an increase in aneuploidy rate with declining embryo quality



Interestingly, fast-growing (grade 5 or 6) aneuploid embryos with a single abnormality appeared to be more often affected by trisomy than monosomy (ratio of monosomies to trisomies 1:1.7), although this did not reach statistical significance (Fisher's exact test, P = 0.1). Slower-growing (grade ≤3) embryos exhibited an almost equal frequency of monosomies and trisomies (ratio of 1.4:1 respectively). Blastocysts with complex (3 or more) chromosome abnormalities were twice as common among embryos displaying slow developmental progression (grade ≤3) compared with fast developing embryos (grade 5 or 6). The increase in complex errors in slower-growing embryos approaches statistical significance , but the difference in comparison with faster growing embryos is too subtle for statistical confirmation in a sample of this size (Fisher's exact test, P = 0.07).

As Figure 4-10 shows, the number of euploid embryos declined with decreasing embryo quality. The opposite was observed for embryos with complex chromosome abnormalities.

4.2.2.4 Morphology at the blastocyst stage and embryo gender

Considering the 806 blastocysts that had a normal sex chromosome complement, males and females occurred at equal frequency, i.e. 393 (48.8%) were male and 413 (51.2%) were female. However, a small excess of male embryos over females (55% vs. 45% respectively) was observed for faster developing blastocysts (grades 5-6). On the contrary, in the case of slower growing (grade ≤3) blastocysts, female embryos predominated over males (53% vs. 47%). It should be noted that neither of these gender differences were statistically significant (Fisher's exact test, P = 0.19 and 0.2, respectively). No correlation was seen between the gender of the blastocyst and the incidence of aneuploidy. These results are shown in Table 4-9.

Table 4-9: The morphology of female and male blastocysts. No differences observed in aneuploidy rate between both genders

Growth rate	Female embryos		Male embryos	
	Normal	Abnormal	Normal	Abnormal
5 or 6	29 (7%)	23 (5.6%)	33 (8.4%)	30 (7.6%)
4	69 (16.7%)	77 (18.6%)	74 (18.8%)	69 (17.6%)
3	78 (18.9%)	85 (20.6%)	58 (14.8%)	74 (18.8%)
< 3	16 (3.9%)	36 (8.7%)	21 (5.3%)	34 (8.7%)
Total	192 (46.5%)	221 (53.5%)	186 (47.3%)	207 (52.7%)
	413 (100%)		393 (100%)	

4.2.2.5 The effect of aneuploidy on the quality of the inner cell mass and trophectoderm

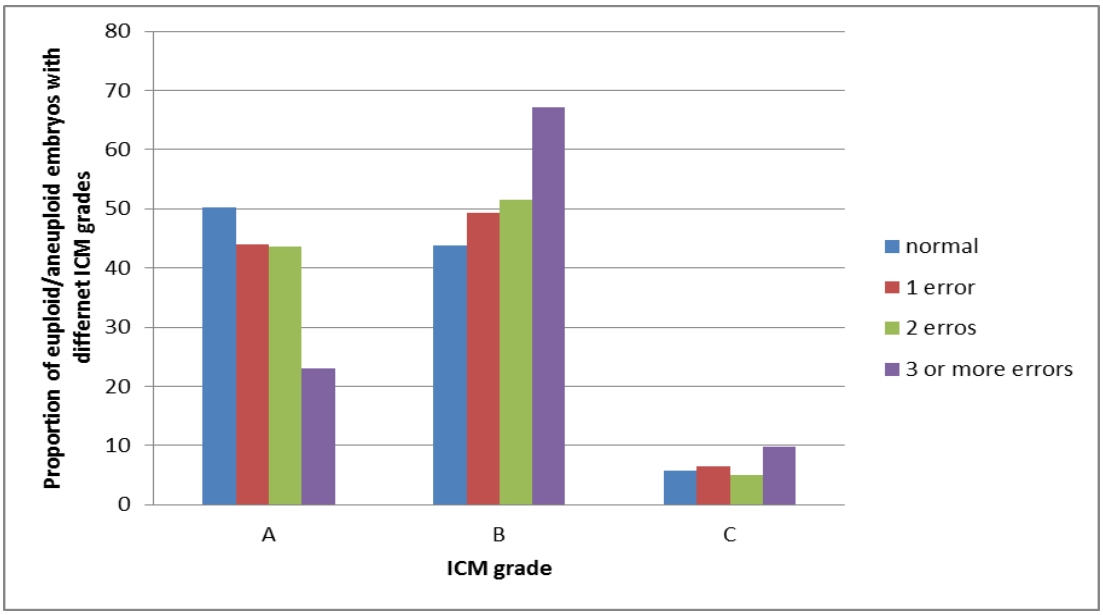
Morphologic evaluation of the ICM revealed that it was significantly affected by the presence of chromosome errors in the embryo (chi square: $P < 0.01$). Specifically, a tightly packed ICM with many cells (grade A) was observed in 50% (172 out of 342) of euploid embryos compared with 40.7% (165 out of 405) of aneuploid embryos. The data obtained, also indicated that ICM quality was highly dependent on the number of chromosome abnormalities. Hence complex aneuploid blastocysts (3 or more errors present) had ICMs of significantly poorer morphology, compared to those of euploid embryos (Fisher's exact test, $P<0.0001$). Table 4-10 and Figure 4-11 demonstrate this data for embryos that formed a full blastocyst and have a distinct ICM and TE layers, i.e. grade ≥ 3.

Table 4-10: ICM morphology in euploid and aneuploid blastocysts. Aneuploidy rate increases with declined ICM quality

ICM Grade	Normal	Abnormal			
		1 error	2 errors	3 or more	Total
A	172 (50.3%)	107 (44%)	44 (43.5%)	14 (22.9%)	165 (40.7%)
B	150 (43.9%)	120 (49.4%)	52 (51.5%)	41 (67%)	213 (52.6%)
C	20 (5.8%)	16 (6.6%)	5 (5%)	6 (9.8%)	27 (6.7%)
Total	342 (100%)	243 (100%)	101 (100%)	61 (100%)	405 (100%)

ICM: Inner cell mass. N/A: non applicable, for embryos that have not formed a full blastocyst

Figure 4-11: The morphology of ICM in relation to chromosomal status showing a decrease in euploidy rate with declining quality



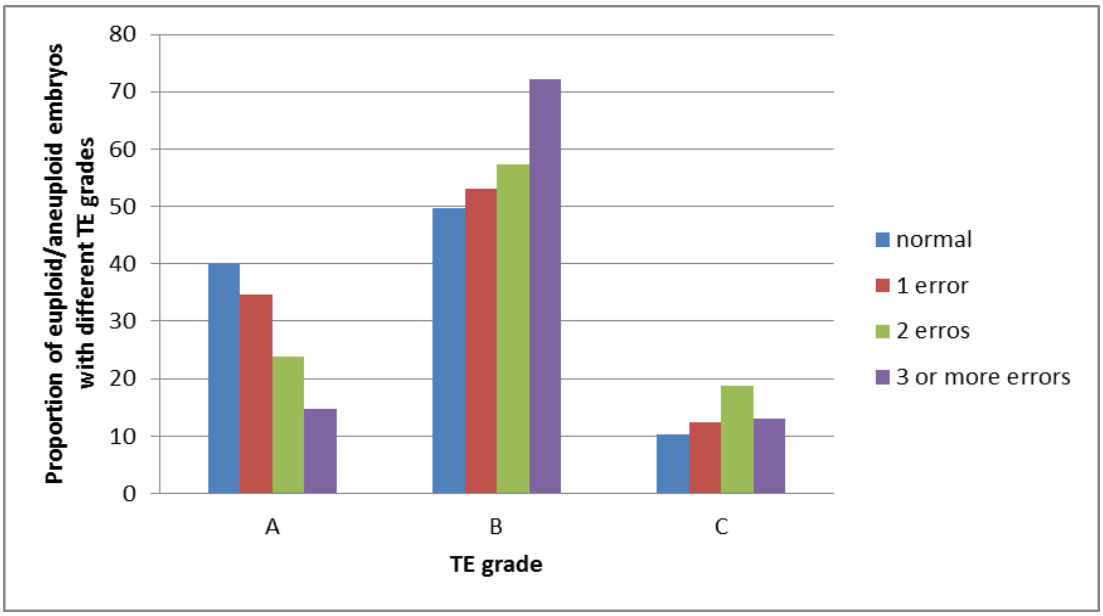
Similar to the ICM observations, the best quality TE (grade A, many cells forming a cohesive epithelium) was associated with euploidy. Specifically, 137/342 (40%) of normal blastocysts had a grade A TE, compared with 117/405 (29%) of those with an aneuploidy, a difference that was statistically significant (Fisher's exact test, P = 0.002). As TE grade declined from A to B to C, there appeared to be an increase in the incidence of aneuploidy involving more than one chromosome, although this was not statistically significant given the sample size. Table 4-11 and Figure 4-12 show this data for embryos that formed a full blastocyst and have a distinct ICM and TE layers, i.e. grade ≥ 3.

Table 4-11: TE morphology in euploid and aneuploid blastocysts. Aneuploidy rate increased with declined TE quality

TE Grade	Normal	Abnormal			
		1 error	2 errors	3 or more	Total
A	137 (40.1%)	84 (34.6%)	24 (23.8%)	9 (14.8%)	117 (28.9%)
B	170 (49.7%)	129 (53.1%)	58 (57.4%)	44 (72.1%)	231 (57%)
C	35 (10.2%)	30 (12.3%)	19 (18.8%)	8 (13.1%)	57 (14.1%)
Total	342 (100%)	243 (100%)	101 (100%)	61 (100%)	405 (100%)

TE: Trophectoderm cells. N/A: non applicable, for embryos that have not formed a full blastocyst

Figure 4-12: The morphology of TE in relation to chromosomal status showing a decrease in euploidy rate with declined TE quality



4.2.2.6 The morphology of blastocysts with aneuploidies at risk of implantation and/or forming a live birth

The morphology and growth rate of aneuploid blastocysts carrying chromosome errors potentially compatible with implantation and, in some cases, live birth (i.e. trisomies 13, 16, 18, 21, 22 and sex chromosome aneuploidies) were compared with those of euploid embryos and blastocysts carrying a single chromosome error not capable of leading to clinically recognised pregnancies (e.g. affecting larger chromosomes). We carried out this comparison so as to investigate whether aneuploid blastocysts capable of producing pregnancies have similar morphology to those that are euploid.

Interestingly, among the best quality and fastest growing embryos (grades 5-6), aneuploid blastocysts carrying an error compatible with clinical pregnancy occurred at a slightly higher frequency than those that were euploid (19.5% and 16.4% respectively, not significant). Aneuploid

blastocysts carrying an anomaly incompatible with a clinical pregnancy formed blastocysts of top morphological quality at a lower rate (11.7%). A breakdown of this data is shown in Table 4-12 and Figure 4-13.

Figure 4-13: Embryo expansion grade, normality and viable and non-viable aneuploidy. No differences observed in embryo quality with different karyotypes

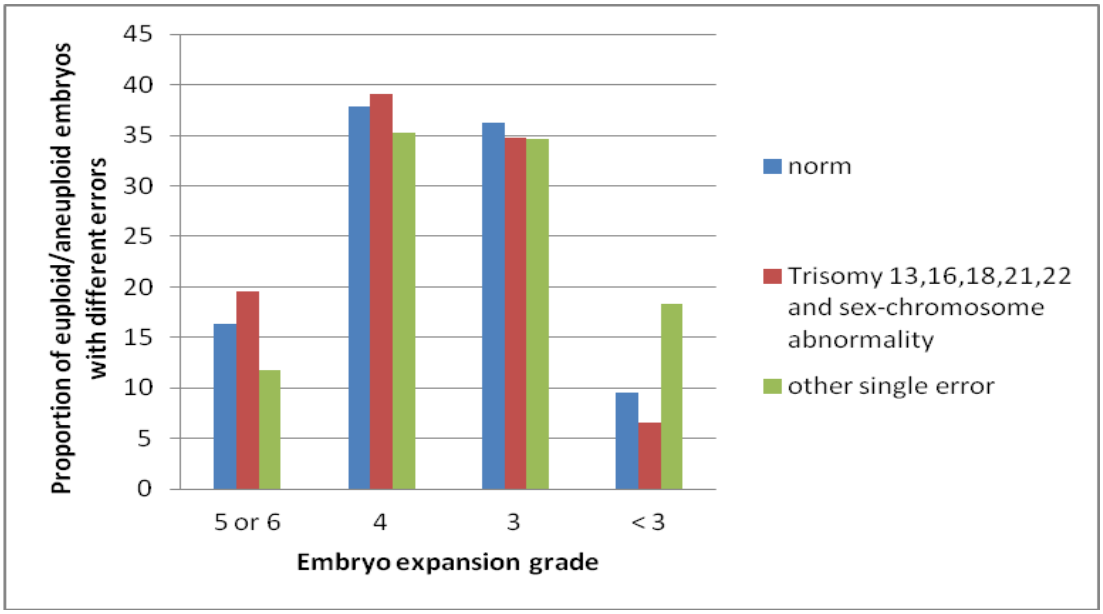


Table 4-12: The morphology of euploid blastocysts, blastocysts with potentially viable aneuploidies, blastocysts with non-viable aneuploidies. No correlation between expansion and different karyotypes

Growth rate	Normal embryos	Abnormal embryos (viable aneuploidies)	Abnormal embryos (non-viable aneuploidies)
5 or 6	62 (16.4%)	18 (19.6%)	23 (11.7%)
4	143 (37.9%)	36 (39.1%)	69 (35.2%)
3	136 (36.2%)	32 (34.8%)	68 (34.7%)
< 3	37 (9.5%)	6 (6.5%)	36 (18.4%)
Total	378 (100%)	92 (100%)	196 (100%)

Regarding the ICM and TE, comparisons between normal blastocysts, blastocysts with viable aneuploidies and ones with non-viable errors showed that there were no significant differences in their morphology among any of these 3 groups (Tables 4-13 and 4-14, and Figure 4-14 A and B).

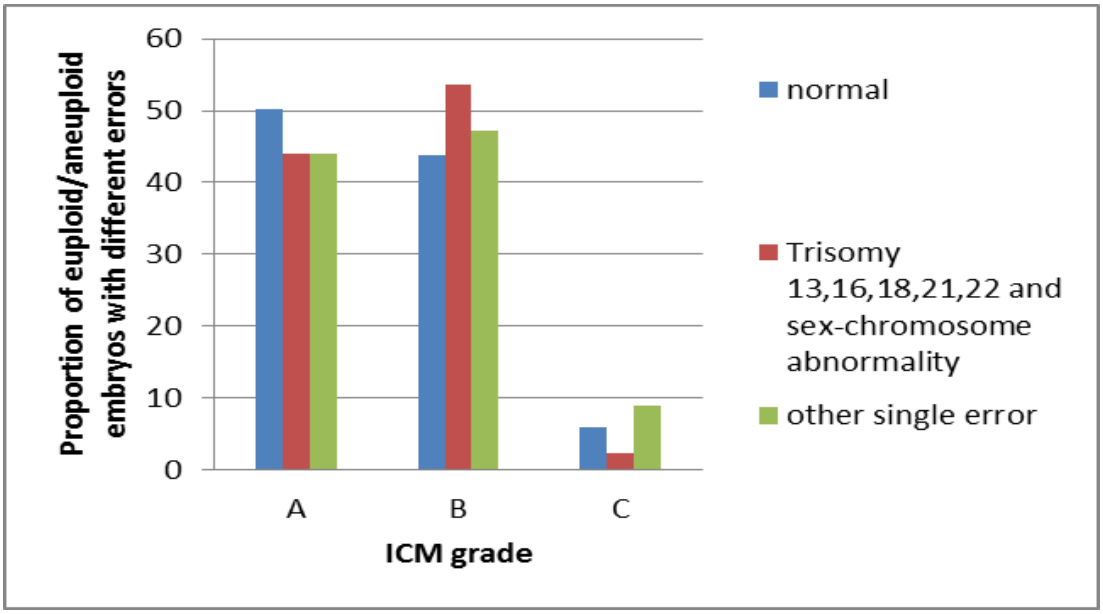
Table 4-13: The ICM morphology of euploid blastocysts, blastocysts with viable aneuploidies, blastocysts with non-viable aneuploidies. No correlation between ICM quality and different karyotypes

ICM morphology	Normal embryos	Abnormal embryos (viable aneuploidies)	Abnormal embryos (non-viable aneuploidies)
A	172 (50.3%)	37 (44%)	70 (44%)
B	150 (43.9%)	45 (53.6%)	75 (47.2%)
C	20 (5.8%)	2 (2.4%)	14 (8.8%)
Total	342 (100%)	92 (100%)	196 (100%)

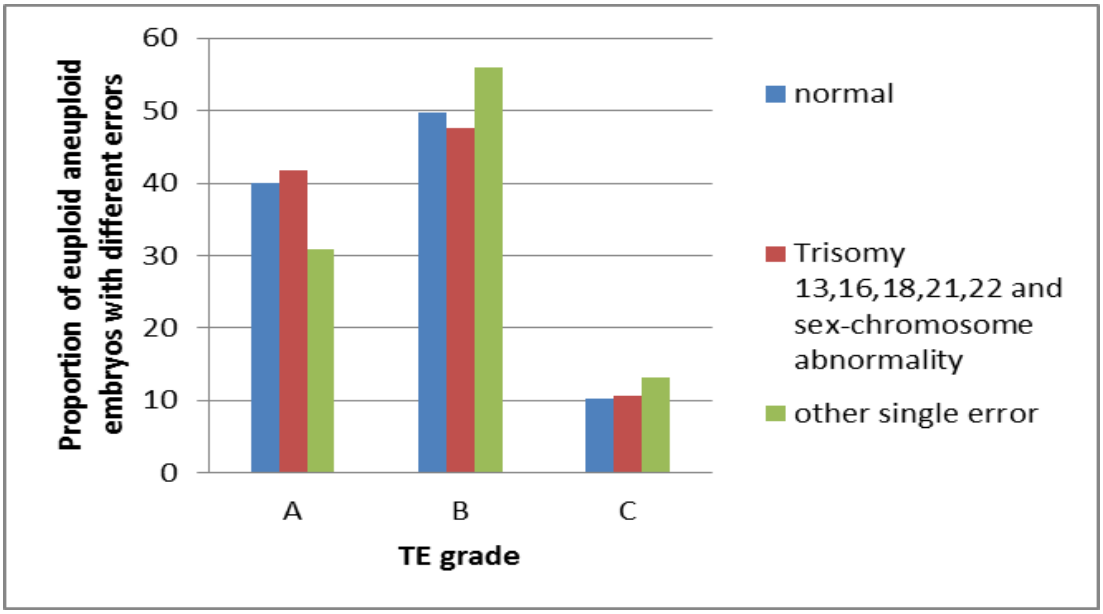
Table 4-14: The TE morphology of euploid blastocysts, blastocysts with viable aneuploidies, blastocysts with non-viable aneuploidies. No correlation between TE quality and different karyotypes

TE morphology	Normal embryos	Abnormal embryos (viable aneuploidies)	Abnormal embryos (non-viable aneuploidies)
A	137 (40.1%)	35 (41.2%)	49 (30.8%)
B	170 (49.7%)	40 (47.6%)	89 (56%)
C	35 (10.2%)	9 (10.7%)	21 (13.2%)
Total	342 (100%)	92 (100%)	196 (100%)

Figure 4-14: A) ICM morphology of normal blastocysts, blastocysts with viable aneuploidies, blastocysts with non-viable aneuploidies. B) TE morphology of normal blastocysts, blastocysts with viable aneuploidies, blastocysts with non-viable aneuploidies. No differences observed between ICM and TE qualities and different karyotypes



A



B

4.3 Summary of findings

According to our data, no correlation was observed between the morphology of three hundred and eighty seven embyros at the cleavage stage and their chromosomal complement apart from highly degraded embryos where an over-representation of aneuploidy was seen. However, a weak correlation was observed between blastocyst morphology and the chromosomal composition of embryos, aneuploidy rate increasing with declining embryo quality. Interestingly, female embryos were found have more cells at the cleavage stage, whereas at the blastocyst stage this trend was reversed.

Of note, embryos with aneuploidies compatible with formation of an ongoing clinical pregnancy (i.e. trisomy 13, 18 or 21 or sex-chromosome abnormalities) had morphologies indistinguishable from those of normal embryos. The only possible way of detecting such embryos prior to implantation remains embryo biopsy followed by chromosomal analysis.

5. Preimplantation genetic diagnosis of chromosome rearrangements using comprehensive molecular cytogenetic methodology

5.1 Aims

Carriers of chromosome rearrangement, such as reciprocal or Robertsonian translocations, may suffer from decreased fertility and represent around 5% of couples with a history of recurrent miscarriage or repeated implantation failure cases (Campana et al, 1986; Frynes and Buggenhout, 1998; Stern et al, 1999). Up until recently, FISH was the method of choice for distinguishing between balanced and unbalanced embryos during PGD of chromosome rearrangements. Although widely applied and successful for this purpose, FISH has two major drawbacks, Firstly, many cases require the development and validation of patient-specific protocols, adding to the cost of the procedure and creating a significant delay before patients can receive treatment. Secondly, FISH is only capable of examining a limited number of chromosomes additional to those involved in the rearrangement (Conn et al, 1998; Munné et al, 1998b; Escudero et al, 2001; Simopoulou et al, 2003; Sampson et al, 2004; Lim et al, 2008; Fischer et al, 2010).

The principal aim of this part of the research project was to evaluate the suitability of comprehensive molecular cytogenetic methods, such as aCGH, for the PGD of different chromosome rearrangements. Additionally, we wanted to test the hypothesis that the presence of chromosome rearrangement might cause malsegregation of structurally normal chromomoses, i.e. not involved in the rearrangement, or what is known as the interchromosomal effect (ICE).

5.2 Results

Overall, molecular karyotypes were obtained for 93% (257/277) of the investigated samples. The only samples that failed to yield results were those for which WGA had been unsuccessful. Both metaphase CGH and aCGH yielded results on all occasions where the DNA of single cells or small groups of cells was amplified successfully.

5.2.1 Cytogenetic analysis of tested samples

Of the samples with molecular karyotypes, 48 (18.7%) were characterised as chromosomally normal (3 PBs, 20 blastomeres, 25 TE samples) and 209 (81.3%) were aneuploid (21 PBs, 127 blastomeres, 61 TE samples). Of these 209 samples, 49 (23.4%) had abnormalities affecting the chromosomes involved in the rearrangement only, whereas for an additional 79 (37.8%) anomalies affecting chromosomes involved in the rearrangement as well as others were scored. The remaining 81 (38.8%) embryos classified as aneuploid were seen to be normal/balanced for the rearrangement in question, but errors affecting other chromosomes were observed (Figure 5-1).

Fifteen of the 39 investigated patients (2, 4, 9, 11, 12, 14, 22, 23, 26, 28, 29, 30, 32, 36 and 38) had no normal embryos for transfer, while the other 24 generated at least one normal embryo (after one or more cycles) and had an embryo transfer. The number of normal and abnormal embryos along with their molecular karyotypes is provided in Table C-1 in the Appendix C. Figure 5-2 represents an example of metaphase CGH analysis of an inversion on chromosome 5 and Figure 5-3 represents an example of aCGH analysis of a reciprocal translocation involving chromosomes 11 and 22.

The smallest imbalance detected in the current study was 2.8 Mbp in size (involved fragment of chromosome 22; 22q11.1-q11.21) as seen in Figure 5-3. The minimum number of micro-

array probes used to diagnose loss or gain of a chromosomal fragment was 15 continuous probes, although in most cases chromosome rearrangements affected a far greater number of clones.

Figure 5-1: Proportion of embryos generated by carriers of chromosome rearrangements with normal and abnormal chromosome complement

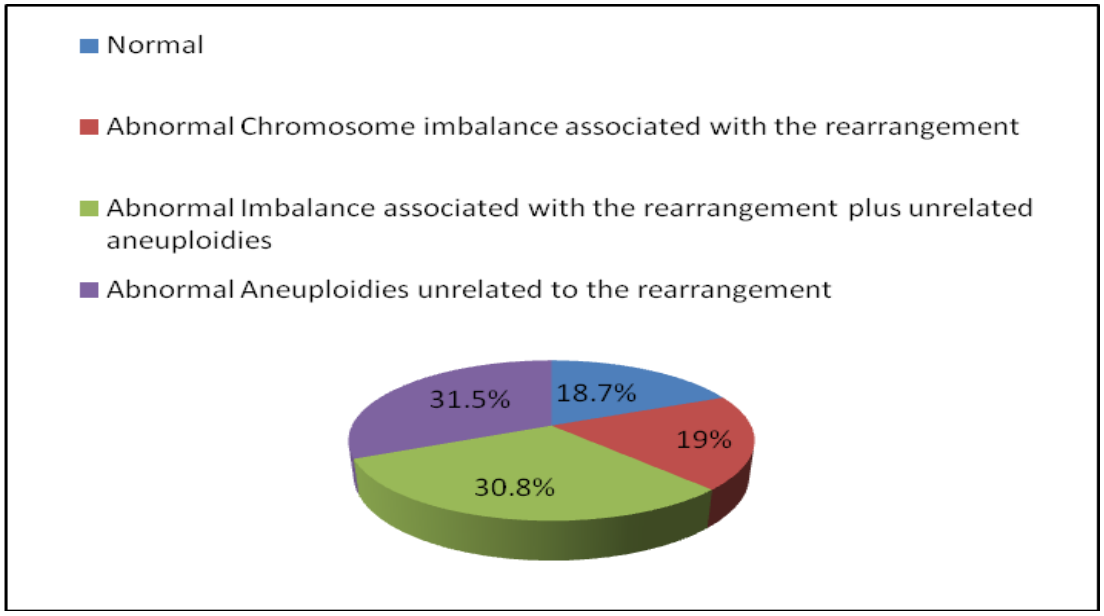
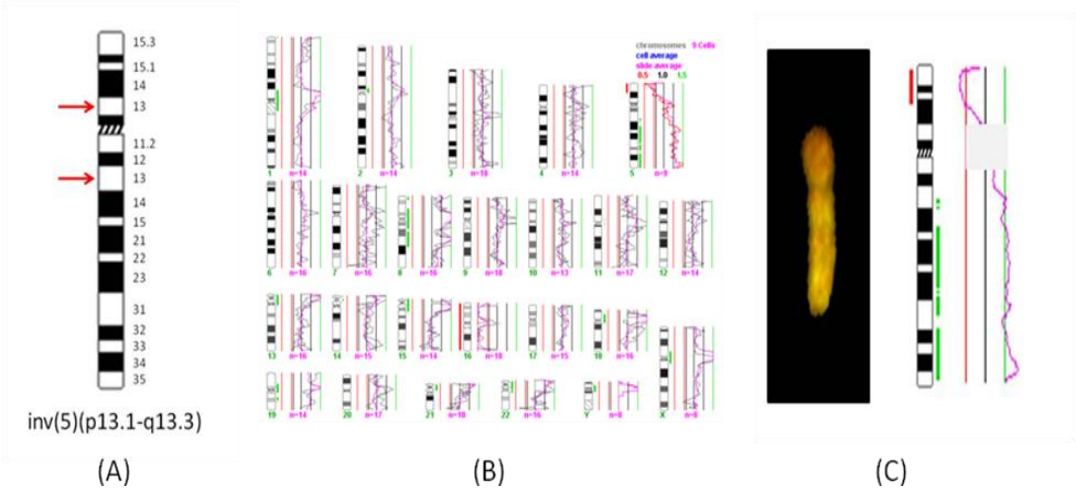
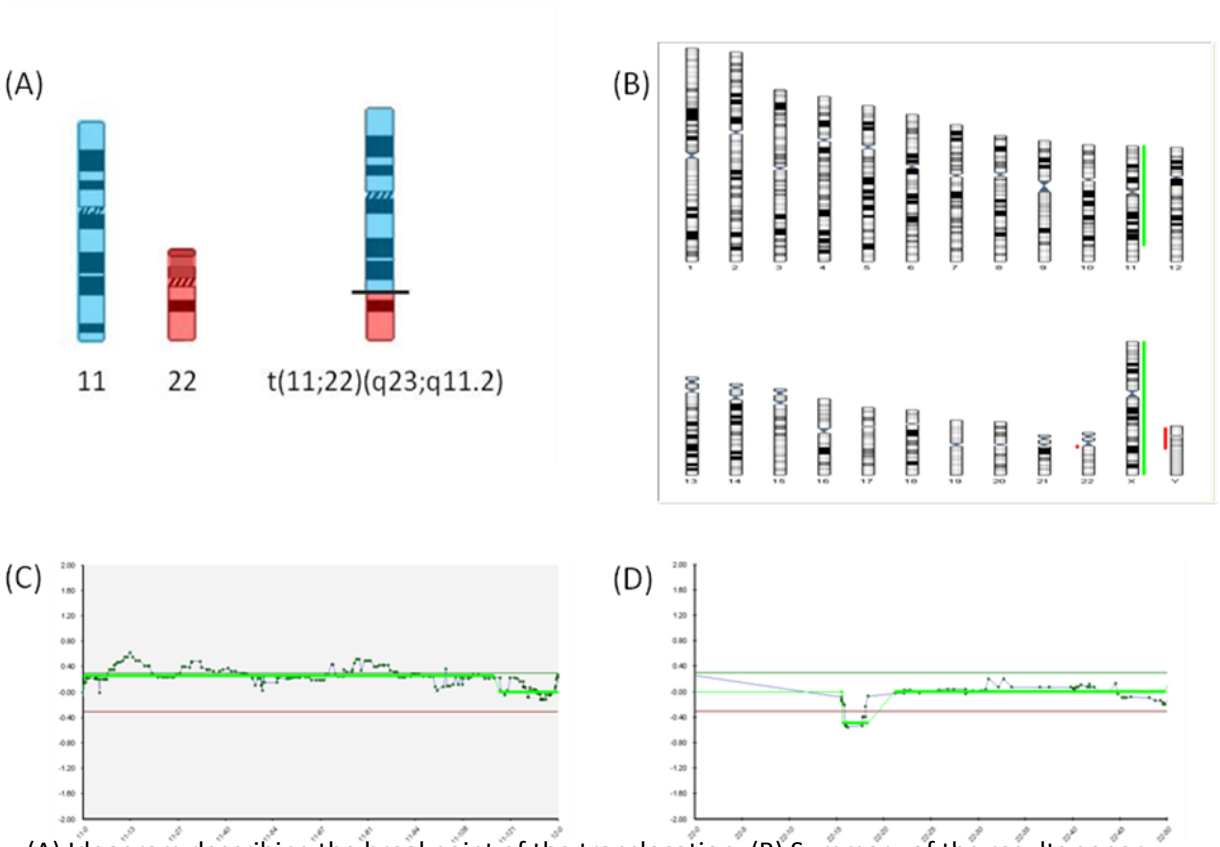


Figure 5-2: Metaphase CGH analysis of a 2nd polar body biopsy from a carrier of an inversion (case 47)



(A) Ideogram describing the breakpoints of the inversion on chromosome 5. (B) Summary of the results generated by the analysis software revealing a loss of a part of the short arm of chromosome 5 (p) and a gain of the long arm (q) along with a loss of chromosome 16. (C) Image of chromosome 5 with hybridized red (reference) and green (sample) DNAs. The red:green flurescence ration along the length of the chromosome displayed on the right. The black line in the middle indicates a 1:1 ratio of red:green fluorescence, the green line represents a 1:1.2 ratio (gain) and the red line indicates a 0.8:1 ratio (loss)

Figure 5-3: Microarray CGH analysis of trophectoderm biopsy from a carrier of a reciprocal translocation (case 13)



(A) Ideogram describing the breakpoint of the translocation. (B) Summary of the results generated by the analysis software revealing a gain of part of chromosome 11 and loss of a part of chromosome 22. (C) Results of the microarray probe along the length of chromosome 11. An excess of material from 11p15.5-11q23 is presented by a clear of all probes in this area (most probes with log2 ratios >0.4. (D) Results from chromosome 22 revealing a loss of material from 22q11.11-q11.12.

5.2.2 Embryo follow up analysis

A small number (23) of the abnormal embryos analysed during this part of the project, characterised aneuploid via metaphase CGH or aCGH, were fixed on glass slides on days 4-6 of preimplantation development and further examined with the use of FISH. The chromosomes involved in the rearrangement were investigated, along with others, including 13, 16, 18, 21 and 22. The results obtained from this re-analysis are shown in Table 5-1. Although chromosomal mosaicism was observed for 7 of these embryos, the accuracy of the initial clinical diagnosis was not affected (i.e. embryos originally diagnosed with an imbalance remained abnormal).

Table 5-1: Result obtained from the re-analysis for a subset of embryos showing confirmation of metaphase CGH/aCGH results using FISH analysis of rearranged chromosomes

Patient	Case	Original CGH/aCGH result	Abnormalities detected by FISH	Concordant with original diagnosis
3	5	46,XX	Normal for chromosomes tested (10); Tetraploid (1); 1 x 18 (1)	Yes
		47,XY,+15	Normal for chromosomes tested (16)	Yes
		45,XY,-15	Normal for chromosomes tested (18); 3 x 18 (2); 1 x 18 (1)	Yes
		45,XX,-14	1 x 14 (32); 1 x 14, 1 x 21 (2); Tetraploid, 2 x 14 (2)	Yes
		46,XX	Normal for chromosomes tested (10) ;Tetraploid (1)	Yes
		46,XX	Normal for chromosomes tested (13)	Yes
8	11	47,XX,-4, +13, +14, -15, +16	3 x 13, 3 x 14, 3 x 16 (3); 3 x 13, 3 x 14 (1); 1 x 13, 3 x 14, 3 x 16, 1 x 18 (1)	Yes
10	13	47,XX,-11q23-qter,+22q11.2-22qter	2 × 11 [cen], 1 × 11 [tel], 3 × 22 [tel] (7)	Yes
		45,XX,+11q23-qter,-22q11.2-22qter	2 × 11 [cen], 3 × 11 [tel], 1 × 22 [tel] (52); 2 × 11 [cen], 2 × 11 [tel], 1 × 22 [tel], 1 × 21 (2); 2 × 11 [cen], 3 × 11 [tel], 0 × 22 [tel], 1 × 16 (1); Tetraploid, 6 × 11 [tel], 2 × 22 [tel] (1)	Yes
		45,XY,+11q23-qter,-22q11.2-22qter	2 × 11 [cen], 3 × 11 [tel], 1 × 22 [tel] (26)	Yes
		46,XX,-11q23-qter,-18,+22q11.2-22qter	2 × 11 [cen], 1 × 11 [tel], 3 × 22 [tel], 1 × 18 (11); 2 × 11 [cen], 1 × 11 [tel], 3 × 22 [tel], 1 × 18, 1 × 21 (1)	Yes
		45,XX,-11pter-11q23,+11q23-11qter	1 × 11 [cen], 2 × 11 [tel], 2 × 22 [tel] (9)	Yes
		45,XX,-18	2 × 11 [cen], 2 × 11 [tel], 2 × 22 [tel], 1 × 18 (20)	Yes
11	14	46,XY,-13,+22	1 × 13, 3 × 22 (4)	Yes
		45,XY,-4	Normal for chromosomes tested (27) Tetraploid (6)	Yes

		45,XY,-13	1 × 13 (5); 1 × 13, 3 × 16 (5); 1 × 13, 1 × 16, 1 × 18 (1); 0 × 13, 1 × 16, 1 × 18, 1 × 22 (1)	Yes
		47,XX,+22	3 × 22 (16); 1 × 22 (7)	Yes
		45,XX,-13	1 × 13 (11)	Yes
		47,XY,+16	3 x 16 (11)	Yes
		46,XY,-10,+15	3 x 15 (32); 3 x 15, 1 x 13 (1)	Yes
		45,XY,-13	1 x 13 (4)	Yes
		45,XX,-13	1 x 13 (12)	
		47,XX,+1	Normal for chromosomes tested (20)	Yes

The number in parentheses in the FISH result column indicates the number of cells with this specific result. [cen]: centromeric probe. [tel]: telomeric probe.

5.2.3 IVF/PGD cycle outcome

Of the 31 patients who have completed their treatment cycles (39 completed cycles), 21 cycles (53.8 %) had no normal embryos and therefore no transfer was performed. In three cycles (7.7%) normal/balanced embryos were detected but no transfer took place due to developmental arrest (patients 6, 35 and 37). Nine cycles (23.1%) had a transfer of normal/balanced embryos, without a pregnancy being achieved. Six cycles (15.4%) achieved a pregnancy, of which one was biochemical, one is still ongoing, and four have resulted in healthy births. Some patients had more than one cycle and for the first cycle they either did not have any normal embryos to be transferred or did not get pregnant after transferring normal embryos.

5.2.4 Frequency of aneuploidy in rearrangement carriers compared to control group

The segregation patterns of >10,000 chromosomes not involved in the rearrangements for which these patients were undergoing PGD were assessed in the 257 samples generated from rearrangement carriers (Table 5-2). Patient 9 was excluded from the statistical analysis as the male partner was a carrier of a reciprocal translocation and the female partner carrier of an inversion, potentially confusing attempts to assess association between specific types of parental rearrangement and the cytogenetics of the embryos produced. However, anecdotally it may be interesting to note that the embryos produced by this couple had unusually high levels of aneuploidy.

Table 5-2: Number of embryos from each cycle and the number of chromosomes assessed along with abnormalities detected

Patient	**Case**	**Maternal age**	**Type of rearrangement**	**Stage tested**	**Embryos with a result**	**Number of chromosomes assessed***	**Number of errors detected****
1	1	29	Rec	Bla	7	294	7
	2	30	Rec	Bla	11	462	6
2	3	30	Rec	Bla	5	210	17
3	4	31	Rob	TE	13	546	9
	5	31	Rob	TE	9	378	2
4	6	31	Rec	TE	3	126	0
5	7	31	Rob	TE	2	84	4
6	8	32	Rob	Bla	4	168	10
4	9	32	Rec	Bla	5	210	13
7	10	32	Rec	TE	7	294	13
8	11	33	Rob	Bla	2	84	4
9	12	33	Rec/Inv	Bla	8	320	37
10	13	33	Rec	TE	9	378	2

11	14	34	Rob	TE	11	462	7
12	15	34	Rec	Bla	3	126	2
13	16	34	Rob	Bla	7	294	22
14	17	34	Rec	Bla	3	126	1
15	18	34	Rec	TE	5	210	3
16	19	35	Rec	Bla	2	84	2
17	20	35	Rec	Bla	5	210	5
18	21	35	Rec	Bla	3	126	24
19	22	35	Rob	Bla	7	294	23
20	23	35	Rec	TE	5	210	1
	24	35	Rec	TE	4	168	2
21	25	36	Inv	TE	3	132	1
22	26	36	Rec	Bla	3	126	4
23	27	36	Rob	Bla	2	84	10
24	28	36	Rec	Bla	4	168	3
25	29	36	Rob	Bla	5	210	18
26	30	36	Rob	Bla	8	336	43
18	31	36	Rec	TE	2	84	0
27	32	37	Rob	Bla	6	252	22
	33	37	Rob	Bla	5	210	5
28	34	37	Rec	Bla	9	378	29
29	35	37	Rob	Bla	6	252	29
30	36	38	Rob	Bla	2	84	2
31	37	39	Rob	Bla	8	336	27
32	38	39	Rec	Bla	2	84	4
33	39	40	Rec	Bla	7	294	10

34	40	40	Rec	Bla	3	126	3
35	41	41	Rec	TE	3	126	4
36	42	41	Rob	TE	5	210	17
37	43	42	Rob	PB	9	189	17
	44	42	Rob	PB	8	168	16
	45	42	Rob	TE	3	126	4
38	46	42	Rec	Bla	5	210	22
39	47	43	Inv	PB	7	154	12
	48	43	Inv	TE	2	88	2
Totals					257	10,291	520

Rec: reciprocal translocation; Rob: Robertsonian translocation; Inv: Inversion; Bla: Blastomere; PB: polar body; TE: trophectoderm cells. *Chromosomes involved in the rearrangement were not assessed; ** Abnormalities affecting chromosomes involved in the rearrangement were excluded.

The control group for ICE assessment consisted of 4,840 samples (analysis of t >199,000 chromosomes). As mentioned earlier, forty eight out of the 257 samples tested from different rearrangement carriers were chromosomally normal (18.7%) and 81.3% were chromosomally abnormal (due to aneuploidies affecting chromosomes involved in the rearrangement or due to spontaneously occurring errors affecting unrelated chromosomes). In the control group, 34.7% of the samples assessed were found to be normal.

In order to assess the presence of an ICE, the chromosomes involved in the rearrangement in each patient were excluded from analysis in both patient and corresponding control groups, thereby focusing the evaluation on chromosomes not involved in the rearrangement (Table 5-3).

Table 5-3: Cytogenetic results obtained from embryos of the patient-matched control group

Matched to case	Age	Stage tested	Number of patients	Embryos with a result	Number of chromo-somes assessed*	Number of errors detected**
1	29	Bla	7	69	2898	136
2	30	Bla	5	56	2352	62
3	30	Bla	5	56	2352	57
4	31	TE	4	27	1134	21
5	31	TE	4	27	1134	21
6	31	TE	4	27	1134	20
7	31	TE	4	27	1134	21
8	32	Bla	10	87	3654	169
9	32	Bla	10	87	3654	166
10	32	TE	5	48	2016	31
11	33	Bla	10	93	3906	144
12	33	Bla	10	93	3720	140
13	33	TE	9	48	2016	27
14	34	TE	13	85	3570	44
15	34	Bla	18	167	7014	383
16	34	Bla	18	167	7014	383
17	34	Bla	18	167	7014	380
18	34	TE	13	85	3570	46
19	35	Bla	17	144	6048	336
20	35	Bla	17	144	6048	337
21	35	Bla	17	144	6048	291
22	35	Bla	17	144	6048	293
23	35	TE	8	63	2646	42
24	35	TE	8	63	2646	42
25	36	TE	13	86	3784	76
26	36	Bla	14	120	5040	286

27	36	Bla	14	120	5040	282
28	36	Bla	14	120	5040	277
29	36	Bla	14	120	5040	282
30	36	Bla	14	120	5040	282
31	36	TE	13	86	3612	73
32	37	Bla	17	122	5124	289
33	37	Bla	17	122	5124	277
34	37	Bla	17	122	5124	290
35	37	Bla	17	122	5124	276
36	38	Bla	16	98	4116	321
37	39	Bla	16	108	4536	227
38	39	Bla	16	108	4536	240
39	40	Bla	16	236	9912	484
40	40	Bla	16	236	9912	500
41	41	TE	21	120	5040	129
42	41	TE	21	120	5040	126
43	42	PB	12	65	1365	148
44	42	PB	12	65	1365	148
45	42	TE	16	76	3192	75
46	42	Bla	17	123	5166	388
47	43	PB	10	61	1342	137
48	43	TE	11	46	2024	96
Totals				4840	199408	9301

Bla: Blastomere; PB: polar body; TE: trophectoderm cells; *Chromosomes involved in the matching patient's rearrangement were excluded from the assessed chromosomes ** Abnormalities affecting chromosomes involved in the matching patient's rearrangement were excluded.

A total of 10,291 chromosomes were examined and 520 aneuploidies detected in the samples generated from the rearrangement carriers. Thus, on average there was a 0.051 proba-

bility of any given chromosome having undergone malsegregation. This error rate was also influenced by female age, while some chromosomes displayed a greater tendency to be abnormal than others. In the control group 199,408 chromosomes were assessed in 4,840 samples (191 polar bodies, 3,615 cleavage stage embryos and 1,034 blastocysts). This analysis led to the detection of 9,301 malsegregation events (0.047 probability of aneuploidy per chromosome per sample). Although small, the increase in the risk of aneuploidy affecting structurally normal chromosomes (0.4% increase per chromosome per sample) was statistically significant (Chi square test, $P < 0.0001$), suggesting the existence of an ICE. A detailed breakdown of aneuploidies detected at each biopsy stage in patient and control samples is given in Table 5-4.

Table 5-4: Malsegregation events detected in non-rearranged chromosomes in patient and control groups. An increase in the aneuploidy rate is observed at the cleavage-stage.

Sample type	**Patient samples**		**Control samples**	
	Segregations	**aneuploidies**	**segregations**	**aneuploidies**
Polar bodies (mean age 42.3)	511	45 (8.8%)	4072	433 (10.6%)
Blastomeres (mean age 35.4)	6158	404 (6.6%)	151644	7978 (5.3%)
Trophectoderm cells (mean age 35.4)	3622	71 (2%)	43692	890 (2%)
Total (mean age 35.7)	10291	520 (5%)	199408	9301 (4.7%)

5.2.4.1 Correction applied to control samples

In order to assess whether the incidence of abnormalities was the same in the presence of a chromosome rearrangement, the data from patient and control samples were compared statistically (Chi squared test). However, pooling data from multiple patient-specific controls was not straightforward, as some control groups were composed of larger numbers of samples than others. Given the importance of female age in aneuploidy predisposition, the control data could have been inadvertently skewed by including disproportionately large numbers of samples from individuals of specific ages. To overcome this problem, it was ensured that the samples from each patient-specific control were given equal weight to the samples from the matched patient (i.e. control data was transformed to match the sample number of the corresponding patient). In order to accomplish this, the frequency of aneuploid chromosomes per sample in each control group (i.e. total number of errors divided by total number of chromosomes analysed) was initially calculated. This Figure was then multiplied by the number of chromosomes assessed for the corresponding matched patient (after excluding the chromosomes involved in the rearrangement). The result represents the number of chromosome errors that would have been expected in the control group had it consisted of the same number of samples as produced by the matched patient. The expected numbers of aneuploidies for the control group are detailed in Table 5-5.

Table 5-5: Corrected number of errors in the patient-specific control group. This step has been performed to neutralize the effect of increased number of errors in the older patients

Case	Age	N° of patient samples	N° of matched control samples	Number of chromosomes assessed in control*	Number of errors detected in control**	Expected number of errors***	Expected number of normal chromosomes***
1	29	7	69	2898	136	14	280
2	30	11	56	2352	62	12	451
3	30	5	56	2352	57	5	205
4	31	13	27	1134	21	10	533
5	31	9	27	1134	21	7	369
6	31	3	27	1134	20	2	123
7	31	2	27	1134	21	2	82
8	32	4	87	3654	169	8	160
9	32	5	87	3654	166	10	200
10	32	7	48	2016	31	5	287
11	33	2	93	3906	144	3	80
12	33	8	93	3720	140	12	304
13	33	9	48	2016	27	5	369
14	34	11	85	3570	44	6	451
15	34	3	167	7014	383	7	120
16	34	7	167	7014	383	16	280
17	34	3	167	7014	380	7	120
18	34	5	85	3570	46	3	205
19	35	2	144	6048	336	5	80
20	35	5	144	6048	337	12	200
21	35	3	144	6048	291	6	120
22	35	7	144	6048	293	14	280
23	35	5	63	2646	42	3	205
24	35	4	63	2646	42	3	164

25	36	3	86	3784	76	3	129
26	36	3	120	5040	286	7	120
27	36	2	120	5040	282	5	80
28	36	4	120	5040	277	9	160
29	36	5	120	5040	282	12	200
30	36	8	120	5040	282	19	320
31	36	2	86	3612	73	2	82
32	37	6	122	5124	289	14	240
33	37	9	122	5124	290	21	360
34	37	6	122	5124	276	14	240
35	37	5	122	5124	277	11	200
36	38	2	98	4116	321	7	78
37	39	8	108	4536	227	17	320
38	39	2	108	4536	240	4	80
39	40	7	236	9912	484	14	280
40	40	3	236	9912	500	6	120
41	41	3	120	5040	129	3	123
42	41	5	120	5040	126	5	205
43	42	9	65	1365	148	20	171
44	42	8	65	1365	148	18	152
45	42	3	76	3192	75	3	123
46	42	5	123	5166	388	16	195
47	43	7	61	1342	137	16	140
48	43	2	46	2024	96	4	80
Total		257	4840	199408	9301	426	9866

5.2.4.2 Reciprocal translocation carriers compared to control samples

The analysis of data from reciprocal translocation carriers (n=115 samples) compared to the appropriate control group revealed no difference in aneuploidy rate (P = 0.87). Regardless of the stage analyzed samples were examined (polar bodies, cleavage stage or blastocyst stage embryos), no evidence for an ICE was found.

5.2.4.3 Inversion carriers compared to control samples

Similarly, no ICE was apparent in samples from inversion carriers (*P*=0.18). However, the number of samples was considered insufficient to conclusively determine whether or not an ICE exists in patients with this type of rearrangement (n=12 samples).

5.2.4.4 Robertsonian translocation carriers compared to control samples

Comparison of samples from Robertsonian translocation carriers (n=122) with corresponding control groups revealed a highly significant increase in aneuploidy rate (P = 0.0002), indicative of a pronounced interchromosomal effect. However, the stage of development was found to have a profound influence on the risk of chromosomal malsegregation. Subdivision of the data from carriers of Robertsonian translocations revealed that an ICE only exists at the cleavage stage (P < 0.0001). No ICE was detected in polar bodies (i.e. oocytes) or TE samples biopsied from blastocysts (P = 0.46 and P = 0.25 respectively).

The number of abnormalities affecting each chromosome was compared between samples from carriers of a Robertsonian translocations and the corresponding control samples (abnormalities affecting chromosomes involved in the rearrangement were not included in matched control samples), to investigate whether one or more specific chromosomes are more prone to

aneuploidy (Table 5-6) compared to others. Chi-square test results showed no significant difference in the aneuploidy rate for any chromosome between the carriers of rearrangements and controls (two tailed P value = 1.00), (Figure 5-4).

Figure 5-4: Frequency of abnormalities in samples from carriers of Robertsonian translocation versus those from matching controls

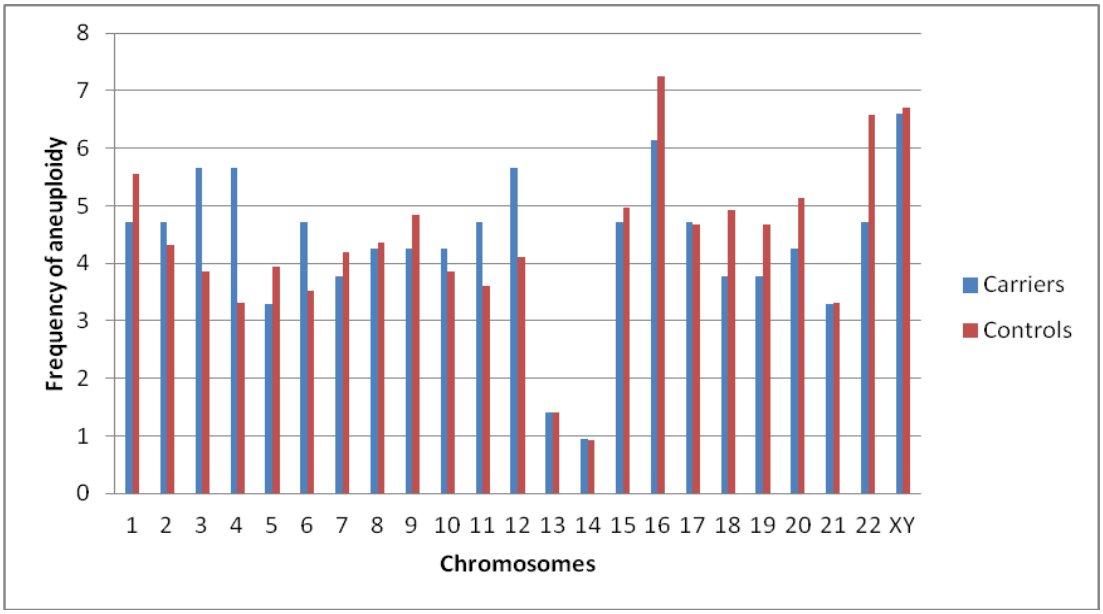


Table 5-6: Number and percentage of aneuploidies affecting each chromosome in carriers of Robertsonian translocations and matching control samples

Chromosome	Robertsonian translocation carrier samples		Matching control samples	
	Number of errors	%	Number of errors	%
1	10	4.716981	131	5.555556
2	10	4.716981	102	4.3257
3	12	5.660377	91	3.859203
4	12	5.660377	78	3.307888
5	7	3.301887	93	3.94402
6	10	4.716981	83	3.519932
7	8	3.773585	99	4.198473
8	9	4.245283	103	4.368109
9	9	4.245283	114	4.834606
10	9	4.245283	91	3.859203
11	10	4.716981	85	3.60475
12	12	5.660377	97	4.113656
13	3	1.415094	33	1.399491
14	2	0.943396	22	0.932994
15	10	4.716981	117	4.961832
16	13	6.132075	171	7.251908
17	10	4.716981	110	4.66497
18	8	3.773585	116	4.919423
19	8	3.773585	110	4.66497
20	9	4.245283	121	5.131467
21	7	3.301887	78	3.307888
22	10	4.716981	155	6.573367
XY	14	6.603774	158	6.700594
Total	212		2358	

5.3 Summary of findings

Microarray CGH provides a universal and accurate tool to analyse embryos generated by carriers of most chromosome rearrangements and is able to detect unbalanced products of segregating rearranged chromosomes. The smallest imbalance detected in the current study was 2.8 Mbp in size (involving a fragment of chromosome 22; 22q11.1-q11.21).

Of the samples with molecular karyotypes 18.7% were characterised as chromosomally normal and 81.3% were aneuploid. Of these 23.4% had abnormalities affecting the chromosomes involved in the rearrangement only, whereas for 37.8% anomalies affecting chromosomes involved in the rearrangement as well as others were scored. The remaining 38.8% of embryos classified as aneuploid were seen to be normal/balanced for the rearrangement in question, but errors affecting other chromosomes were observed.

An interchromosomal effect (ICE) was clearly observed in cleavage-stage embryos from Robertsonian translocation carriers, but not at any other developmental stages. This indicates an effect of chromosome rearrangements on the segregation of structurally normal chromosomes during mitotsis, but does not support the existence of a meiotic ICE.

6. The cytogeneticsand implantation ability of embryos generated by an infertile population

6.1 Aims

Data obtained from the examination of spontaneous abortion material have shown that approximately two thirds of lost clinical pregnancies are chromosomally abnormal (Menahsa et al, 2005). During preimplantation development the incidence of chromosome abnormality is even higher, as revealed by numerous studies performed at the cleavage stage (Delhanty et al, 1993; Munné et al, 1993; Iwarson et al, 1999; Wilton et al, 2003) as well as at the blastocyst stage (Evsikov and Verlinsky, 1998; Ruangvutilert et al., 2000; Sandalinas et al., 2001; Coonen et al., 2004; Bielanska et al., 2005; Daphnis et al., 2005; Li et al., 2005; Fragouli et al, 2010; Schoolcraft et al, 2010; Yang et al, 2012). Although most of these investigations only assessed a limited number of chromosomes per embryo, using FISH, they have nonetheless succeeded in confirming high aneuploidy rates.

The aim of this part of the project was investigate whether there are differences in the frequency and types of chromosome errors seen in embryos generated by an infertile population with different indications or not. Such patients required assisted reproductive treatment in combination with preimplantation genetic screening in order to conceive. The clinical outcomes after these procedures are also discussed.

6.2 Results

6.2.1 Advanced female age patient group

6.2.1.1 Aneuploidy rate

Of the 490 day 3 embryos which were biopsied, 426 (87%) were analysed successfully and 88.3% (376/426) of these embryos were found to be chromosomally abnormal. On the other hand, of the 520 embryos biopsied on day 5, 484 (93.1%) were analysed successfully and 62.6% (296/473) were characterized abnormal. A breakdown of number of errors can be found in Tables 6-4 for cleavage stage embryos and 6-10 for blastocyst stage embryos.

6.2.1.2 Chromosome errors

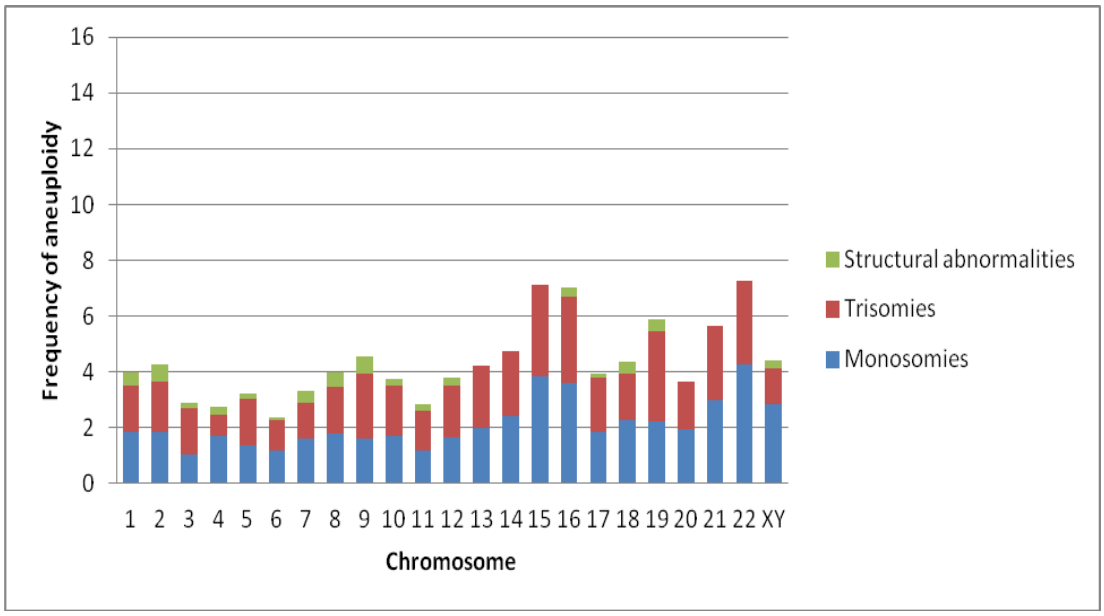
A total of 1,449 chromosome errors were scored in 426 embryos at the cleavage stage. At the blastocyst stage, a total of 611 errors were scored in 484 embryos. A breakdown of errors can be found in Table 6-1 and Figure 6-1. Molecular karyotypes for embryos from this group of patients at the cleavage and blastocyst stages are shown in Tables D-7 and D-8, respectively, in Appendix D.

Table 6-1: Number and type of errors in embryos generated from women of advanced age

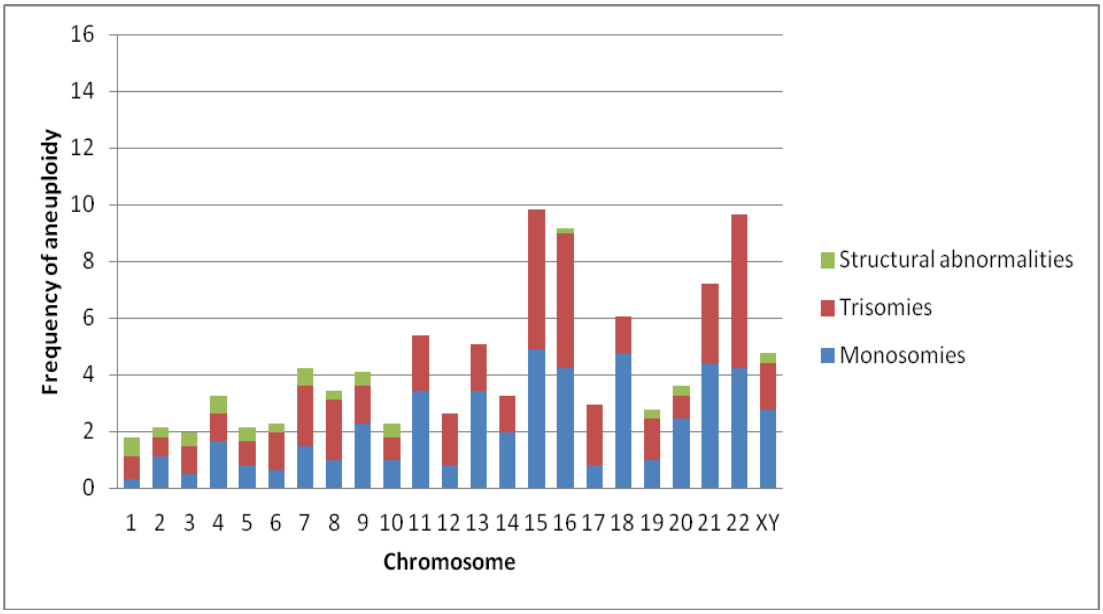
Stage	Number of embryos	Abnormalities		
		Monosomies	Trisomies	Partials*
Cleavage	426	707 (48.8%)	659 (45.5%)	83 (5.7%)
Blastocyst	473	306 (50.1%)	268 (43.9%)	37 (6.1%)

* Refers to aneuploidy that does not involve the entire length of a chromosome – i.e. a segmental aneuploidy, presumably resulting from a structural abnormality/chromosome breakage.

Figure 6-1: Frequency of errors scored in embryos generated from AMA patients:
A) Cleavage stage B) Blastocyst stage



(A)



(B)

6.2.2 Recurrent miscarriages patient group

6.2.2.1 Aneuploidy rates at the cleavage and blastocyst stages

Of the 160 cleavage stage embryos which were biopsied, 145 (90.6%) were analysed successfully and 71.7% (104/145) were characterized abnormal. Additionally, of the 312 blastocysts biopsied on day 5, 290 (93%) were analysed successfully and 53.1% (154/290) were characterized abnormal. A breakdown of number of errors can be found in tables 6-4 for cleavage stage embryos and 6-10 for blastocyst stage embryos.

6.2.2.2 Cytogenetic analysis

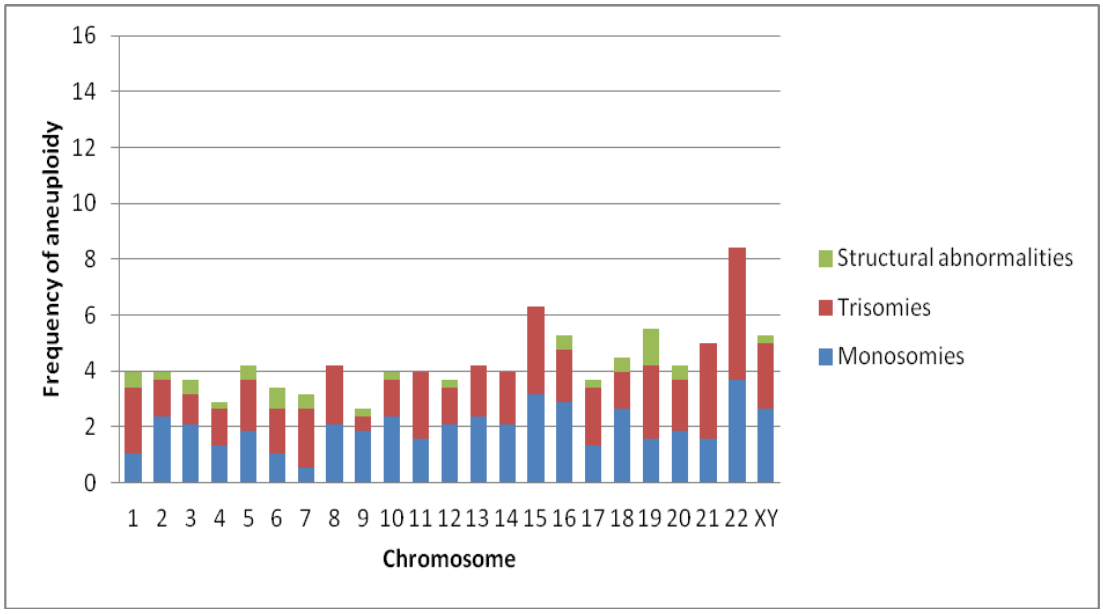
A total of 380 chromosome errors were scored in 145 embryos at the cleavage stage. A total of 260 errors were scored in 290 embryos at the blastocyst stage. A breakdown of errors can be found in Table 6-2 and Figure 6-2. Karyotypes of cleavage and blastocyst stage embryos for recurrent pregnancy loss miscarriage (RM) patients are displayed in Tables D-9 and D-10, respectively, in Appendix D.

Table 6-2: Number and type of errors in embryos generated from patients with unexplained recurrent miscarriages

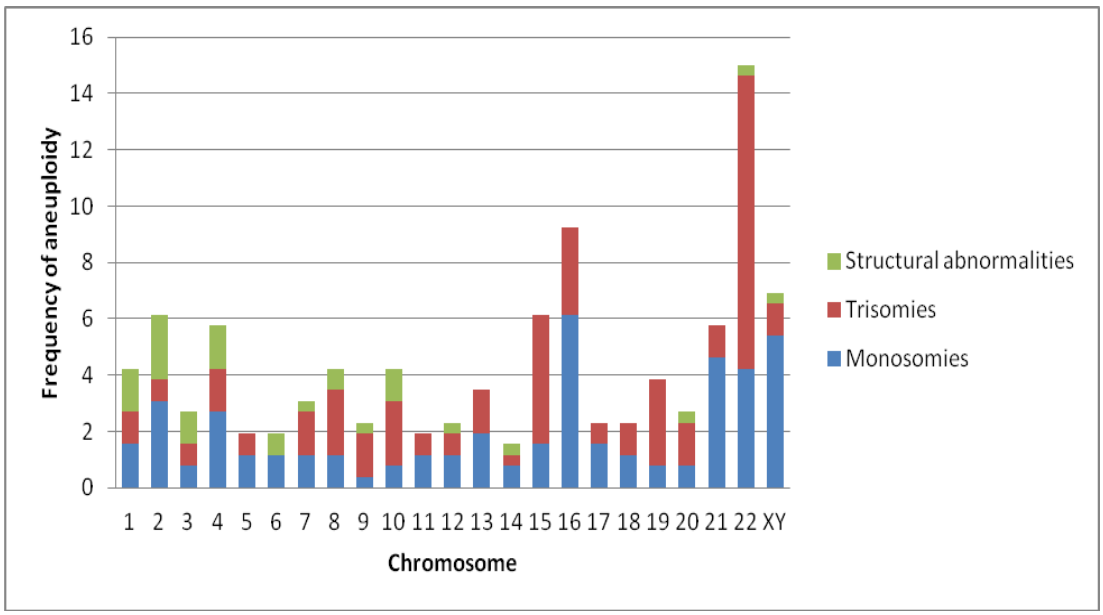
Stage	Number of embryos	Abnormalities		
		Monosomies	Trisomies	Partials*
Cleavage	145	175 (46.1%)	176 (46.3%)	29 (7.6%)
Blastocyst	290	117 (45%)	112 (43.1%)	31 (11.9%)

* Refers to aneuploidy that does not involve the entire length of a chromosome – i.e. a segmental aneuploidy, presumably resulting from a structural abnormality/chromosome breakage.

Figure 6-2: Frequency of errors scored in embryos generated from RM patients:
A) Cleavage stage B) Blastocyst stage



(A)



(B)

6.2.3 Repeated implantation failure patient group

6.2.3.1 Aneuploidy rates at the cleavage and blastocyst stages

A total of 90.3% (130/144) of cleavage stage and 95.1% (252/265) of blastocyst stage embryos generated by patients with repeated implantation failure (RIF) were analysed successfully and 81.5% (106/130) and 54.4% (137/252) were characterized aneuploid, respectively. A breakdown of number of errors can be found tables 6-4 for cleavage stage embryos and 6-10 for blastocyst stage embryos.

6.2.3.2 Cytogenetic analysis

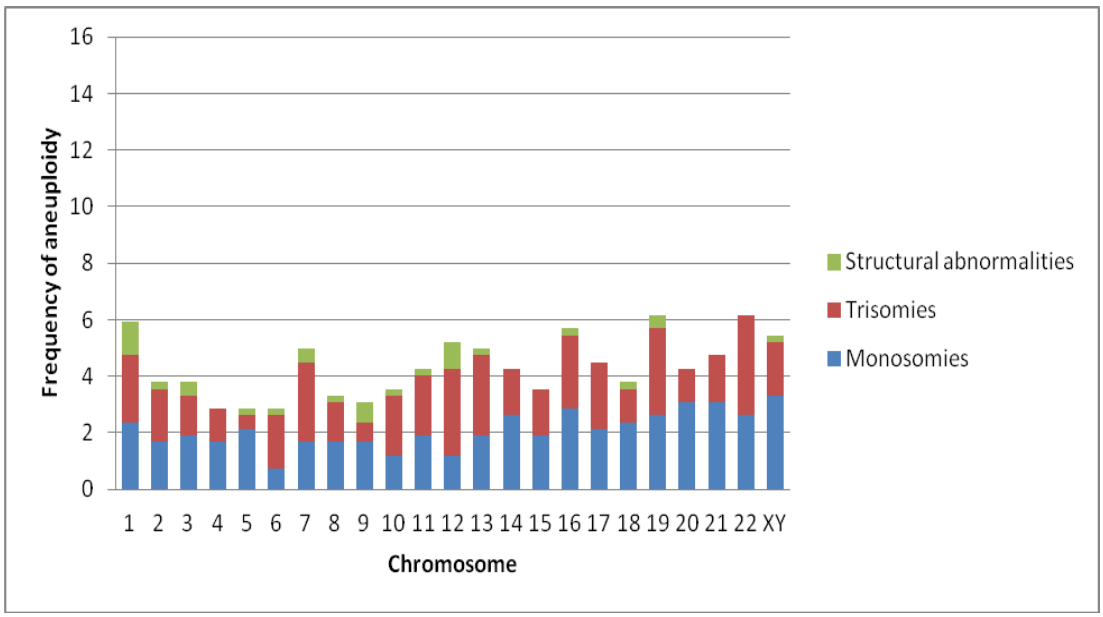
A total of 422 chromosome errors were scored in 130 embryos at the cleavage stage and a total of 205 errors were scored in 252 embryos at the blastocyst stage. A breakdown of errors can be found in Table 6-3 and Figure 6-3. Embryo karyotypes for this group of patient are shown in Tables D-11 (cleavage stage) and D-12 (blastocyst stage) in Appendix D.

Table 6-3: Number and type of errors in embryos generated from RIF patients

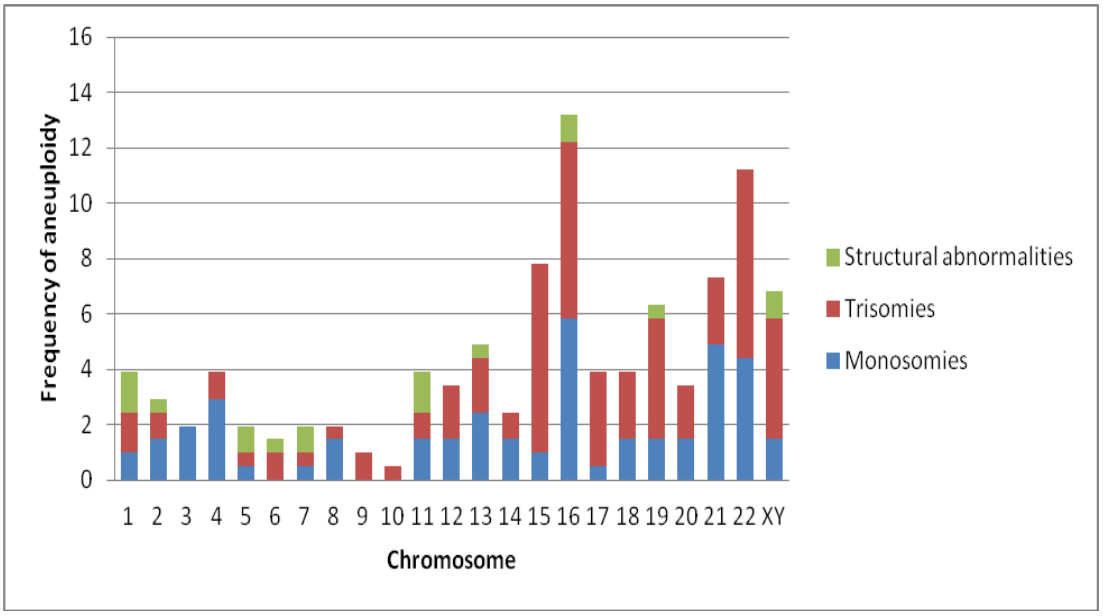
Stage	Number of embryos	Abnormalities		
		Monosomies	Trisomies	Partials*
Cleavage	130	203 (48.1%)	191 (45.3%)	28 (6.6%)
Blastocyst	252	80 (39%)	107 (52.2%)	18 (8.8%)

* Refers to aneuploidy that does not involve the entire length of a chromosome – i.e. a segmental aneuploidy, presumably resulting from a structural abnormality/chromosome breakage.

Figure 6-3: Frequency of errors scored in embryos generated from RIF patients:
A) Cleavage stage. B) Blastocyst stage



(A)



(B)

6.2.4 Analysis of chromosome abnormalities according to preimplantation development stage and PGS indication

6.2.4.1 Cleavage stage

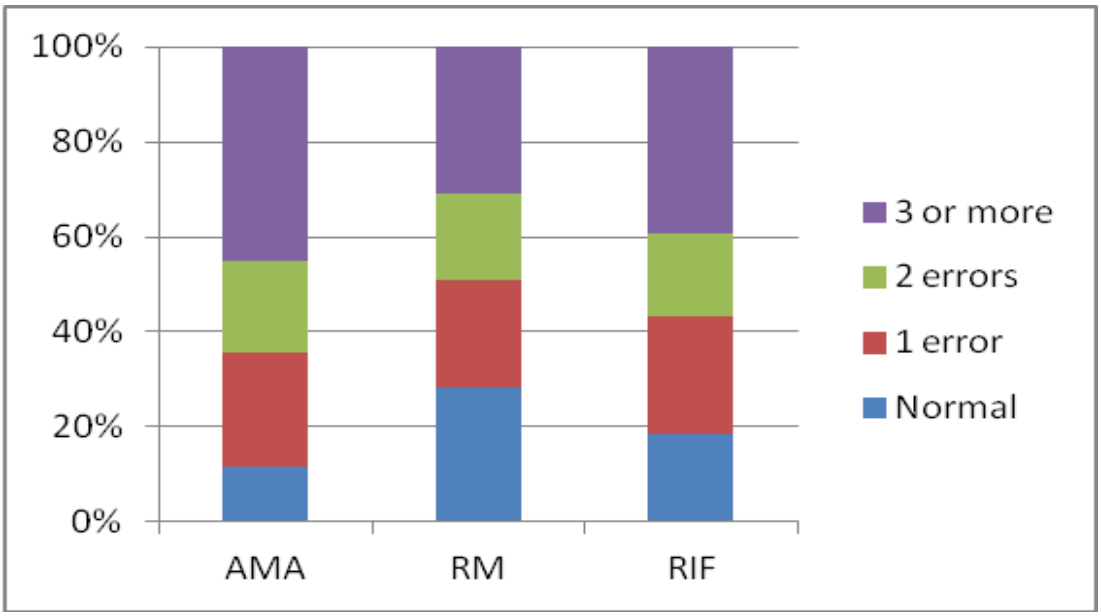
Overall, the aneuploidy rate observed for embryos at the cleavage stage of preimplantation development [83.6% (586/701)] was significantly elevated compared to the blastocyst stage [57% (585/1026)] (Chi squared test, $P < 0.0001$). This rate was seen for an average female age of 39.6 years (age range 27-45 years). High aneuploidy rates were seen for the individual patient groups as well. Specifically, a total of 88.3% of all cleavage stage embryos generated by women of advanced reproductive age (average age 40.8) were chromosomally abnormal. A slightly lower abnormality rate (81.5%) was scored in embryos of RIF couples. However, many of these patients were also of advanced reproductive age (average female age 36.8 years, age range 27-44 years). The lowest incidence of aneuploidy was observed in the embryos of RM couples (aneuploidy rate of 71.7% for an average female age of 37.9 years, age range 31-45 years). The difference in aneuploidy rates observed is statistically significant for AMA and RM patients ($P < 0.0001$), while there was only a tendency towards statistical significance between AMA and RIF and between RIF and RM patients ($P = 0.055$ and 0.065, respectively).

Abnormal embryos were grouped according to the number of errors scored, i.e. 1, 2 or 3 or more abnormalities. These results are presented in Table 6-4, whereas the frequency of normal and the different types of abnormal embryos are presented in Figure 6-4.

Table 6-4: Normal and abnormal cleavage stage embryos generated from AMA, RM and RIF patients. A significant decrease in aneuploidy rate is found in embryos from RM group.

PGS indication	Normal	Abnormal		
		1 error	2 errors	3 or more
AMA	50 (11.7%)	101 (23.7%)	83 (19.5%)	192 (45.1%)
RM	41 (28.3%)	33 (22.8%)	26 (17.9%)	45 (31%)
RIF	24 (18.5%)	32 (24.6%)	23 (17.7%)	51 (39.2%)

Figure 6-4: Frequency of embryos characterized as normal, abnormal with 1 error, 2 errors and 3 or more errors from patients with different indications showing a significant increase in euploid embryos generated from RM patients



No statistical significance was observed between embryos with different numbers of errors between the different groups (Chi squared test, P = 0.7).

The breakdown of individual chromosome error frequency for each of the three patient groups can be found in Table 6-5. The top five chromosomes in terms of aneuploidy rate at the cleavage stage were 22, 15, 16, 19 and 21 for the AMA group, in the embryos from the RM patient group they were 22, 15, 19, X/Y and 16, and in the embryos of the RIF patient group they were 22, 19, 1, 16 and X/Y. The top five most abnormal chromosomes scored in all embryos at the cleavage stage regardless of indication were 22, 16, 15, 19 and 21. No statistical significance was observed between aneuploidy frequencies for different chromosomes in different patient groups except for chromosome 15 which displayed significantly less aneuploidy in the RIF group compared with the AMA patients (Chi square test, P = 0.04).

Table 6-5: Frequency of aneuploidy for each chromosome as a percentage of all abnormalities detected. No significant differences were observed in aneuploidy rate of chromosomes from different patient groups

Chromosome	AMA (%)	RM (%)	RIF (%)
1	4.00	3.95	5.92
2	4.28	3.95	3.79
3	2.90	3.68	3.79
4	2.76	2.89	2.84
5	3.24	4.21	2.84
6	2.35	3.42	2.84
7	3.31	3.16	4.98
8	4.00	4.21	3.32
9	4.55	2.63	3.08
10	3.73	3.95	3.55
11	2.83	3.95	4.27
12	3.80	3.68	5.21
13	4.21	4.21	4.98
14	4.76	3.95	4.27
15	7.11	6.32	3.55
16	7.04	5.26	5.69
17	3.93	3.68	4.50
18	4.35	4.47	3.79
19	5.87	5.53	6.16
20	3.66	4.21	4.27
21	5.66	5.00	4.74
22	7.25	8.42	6.16
XY	4.42	5.26	5.45

The average female age was different for patients of the examined groups, i.e. 40.8 years for AMA, 37.9 years for RM and 36.8 years for RIF. Therefore, the patients from each group were further sub-divided according to female age into younger (36 years or less) and older (37 years or more).

6.2.4.1.1 The incidence of aneuploidy in cleavage stage embryos generated by a "younger" patient group

Embryos generated by RM and RIF couples where the female partner's age was 36 years or less were analysed. Details about the patients and their embryos included in this younger age group are shown in Table 6-6.

The total aneuploidy rate scored for both patient groups (RM and RIF) was 66.7% (70/105) for average female age 33.5 years (age range 27-36 years). The aneuploidy rate in the RM group was 61% (36/59) for average female age 34.2 years (age range 31-36 years) while the aneuploidy rate in the RIF group was 73.9% (34/46) for average female age 32.8 years (age range 27-36 years). No statistical significance was observed in the aneuploidy rate between RM and RIF groups (Chi square test, P = 0.2).

The total number of errors scored in the cleavage stage embryos from the younger patients was 258 errors (163 errors in the RM group and 95 errors in the RIF group).

Table 6-6: Details of the "younger" patient group

	RM	**RIF**
Number of patients	9	8
Average maternal age	34.2 years	32.75 years
Number of embryos	59	46

In this groups of patients, no statistical significance was observed between the incidence of chromosome gains and losses (monosomy:trisomy: 1.1:1 for the RM group and 0.9:1 for the RIF group) (Fisher's exact test, P = 0.55). In addition to the whole chromosome errors, partial abnormalities were scored. These accounted for 9.8% and 9.5% of scored abnormalities in the RM and RIF groups, respectively.

Abnormal embryos were also grouped according to the number of chromosome abnormalities they were carrying (Table 6-7). No statistical significance was observed between the number of embryos with different number of errors from RM or RIF patients and this might be due to the small number of patients in each group (Chi square test, P = 0.32).

Table 6-7: Frequency of normal and abnormal embryos from younger RM and RIF patients. No significant differences observed

PGS Indication	**Normal**	**Abnormal**		
		1 error	**2 errors**	**3 or more**
RM	23 (39%)	12 (20.3%)	5 (8.5%)	19 (32.2%)
RIF	12 (20.1%)	14 (30.4%)	7 (15.2%)	13 (28.3%)

6.2.4.1.2 The incidence of aneuploidy in cleavage stage embryos generated by an "older" patient group

Embryos and patients from all groups (RIF, RM and AMA) were included in this sub-group and their details can be found in Table 6-8. The aneuploidy rate in the AMA group was 88.4% (375/424) for average female age 40.9 years (age range 37-45 years), for the older RM patients aneuploidy rate was 79% (68/86) for average female age 40.3 years (age range 37-45 years), while the aneuploidy rate in the older RIF group was 85.7% (72/84) for average maternal age 40 years (age range 37-44 years). A significant increase was observed in aneuploidy rate between AMA and RM embryos (Chi square test, P = 0.02). However, no statistical significance was observed when comparing RM and RIF groups or AMA and RIF (Chi square test, P = 0.32 and 0.46, respectively).

Table 6-8: Details of the "older" patient group

	AMA	**RM**	**RIF**
Number of patients	72	14	10
Average maternal age	40.9	40.3 years	40 years
Number of embryos	424	86	84

The total number of errors scored in the cleavage stage embryos from the older patients was 1,976 errors (1432 errors in the AMA group, 217 errors in the RM group and 327 errors in the RIF group).

No statistical significance was observed between the incidence of chromosome gains and losses, (monosomy:trisomy: ~1:1 for all groups, i.e.1.06:1, 0.93:1 and 1.11:1 for AMA, RM and RIF patients, respectively) (Chi square test, *P* = 0.61). Partial abnormalities accounted for 5.8%, 6.5% and 5.8% of chromosome errors scored in the AMA, RM and RIF embryos, respectively.

Details about the frequency of normal and abnormal embryos (with a single, double and three or more errors) from this older patient group (AMA, RM and RIF) are shown in Table 6-9. The proportion of embryos with one or two errors was similar for the three groups. A significant increase in the proportion of embryos with 3 or more errors was observed in the AMA and RIF group compared to that for the RM group (Fisher's exact test, P = 0.01 and 0.027, respectively).

Table 6-9: Frequency of normal and abnormal embryos from the older patient group and a significant increase in aneuploidy rate in AMA group compared to the RM group

PGS Indication	**Normal**	**Abnormal**		
		1 error	**2 errors**	**3 or more**
AMA	49 (11.6%)	101 (23.8%)	83 (19.6%)	191 (45%)
RM	18 (21%)	21 (24.4%)	21 (24.4%)	26 (30.2%)
RIF	12 (14.3%)	17 (20.2%)	15 (17.9%)	40 (47.6%)

6.2.4.2 Blastocyst stage

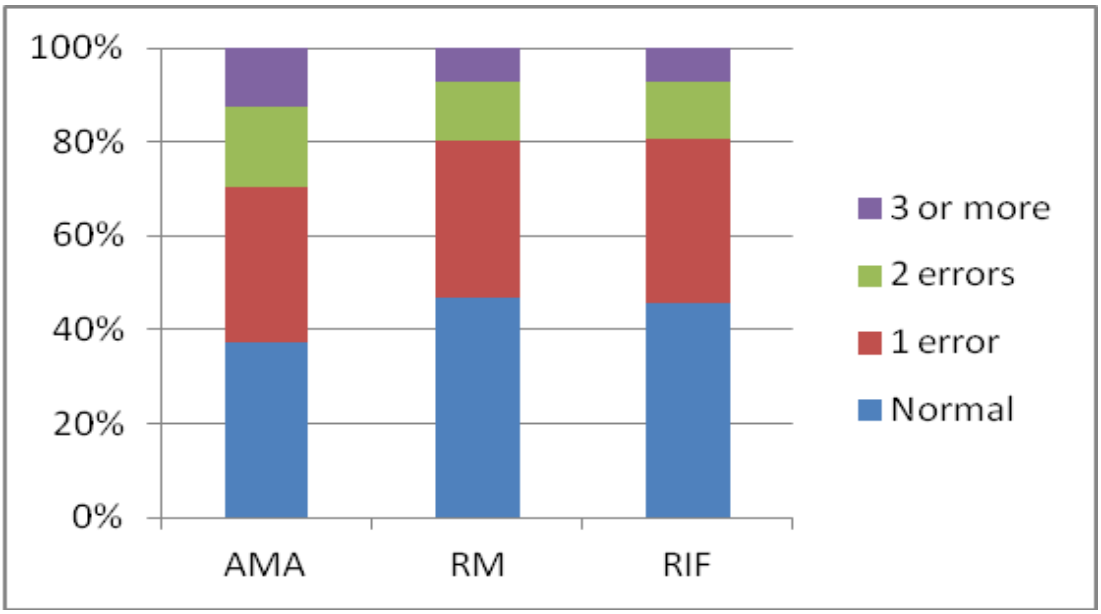
The aneuploidy rate observed for blastocyst stage embryos was 57% (585/1026) and as mentioned earlier had significantly decreased, compared to the cleavage stage (Chi squared test, P <0.0001). This abnormality rate was seen for an average female age of 38.9 years (age range 28-47 years). Lower aneuploidy rates were observed for the three individual patient groups as well. Specifically, a total of 63% (305/484) of blastocysts generated by the AMA patient group [average female age 40.4 years, age range 35-47 years], 54.4% (137/252) of those generated by the RIF patient group [average female age 37.5 years, age range 28-44 years], and 53.1% (154/290) of the RM blastocyst embryos [average female age 36.6 years, age range 30-43 years] were character-ised as abnormal. A significantly higher aneuploidy rate was observed for embryos generated by AMA patients compared to RM and RIF patients (Chi square test, P = 0.01 and 0.03, respectively). No difference was seen between embryos from the RM and RIF groups.

Abnormal embryos were grouped according to the number of errors scored, i.e. 1, 2 or 3 or more abnormalities. These results are presented in Table 6-10, whereas the frequency of normal and the different types of abnormal embryos are presented in Figure 6-5.

Table 6-10: Normal and abnormal blastocyst stage embryos generated from AMA, RM and RIF patients. AMA shows a significant increase in aneuploidy rate compared to RM and RIF

PGS Indication	Normal	Abnormal		
		1 error	2 errors	3 or more
AMA	179 (37%)	159 (32.9%)	85 (17.6%)	61 (12.6%)
RM	136 (46.9%)	97 (33.4%)	36 (12.4%)	21 (7.2%)
RIF	115 (45.6%)	88 (34.9%)	31 (12.3%)	18 (7.1%)

Figure 6-5: Frequency of embryos characterized as normal, abnormal with 1 error, 2 errors and 3 or more errors from patients with different indications. A significant inccrease in aneuuploidy rate observed for embryos from AMA group



The breakdown of individual chromosome error frequency for each of the three patient groups can be found in Table 6-11. The top five chromosomes in terms of aneuploidy scored in blastocysts generated by the AMA patient group were 15, 22, 16, 21 and 18, in the embryos from the RM patient group they were 22, 16, X/Y, 15 and 16 and 2, and in the embryos of the RIF patient group they were 16, 22, 15, 21 and X/Y. No statistical significance was observed between aneuploidy frequencies for different chromosomes in different patient groups apart from a significant increase in the frequency of errors affecting chromosomes 2 and 22 in the RM group was observed compared to AMA group (Fisher's exact test, P = 0.006 and 0.03, respectively).

Table 6-11: Frequency of chromosome abnormalities in the three examined patient groups. A significant increase in the aneuploidy rate was only observed for chromosomes 2 and 22 for the RM group

Chromosome	AMA	RM	RIF
1	1.80	4.23	3.90
2	2.13	6.15	2.93
3	1.96	2.69	1.95
4	3.27	5.77	3.90
5	2.13	1.92	1.95
6	2.29	1.92	1.46
7	4.26	3.08	1.95
8	3.44	4.23	1.95
9	4.09	2.31	0.98
10	2.29	4.23	0.49
11	5.40	1.92	3.90
12	2.62	2.31	3.41
13	5.07	3.46	4.88
14	3.27	1.54	2.44
15	9.82	6.15	7.80
16	9.17	9.23	13.17
17	2.95	2.31	3.90
18	6.06	2.31	3.90
19	2.78	3.85	6.34
20	3.60	2.69	3.41
21	7.20	5.77	7.32
22	9.66	15.00	11.22
XY	4.75	6.92	6.83

The average female age was different for patients of the examined groups, i.e. 40.4 years for AMA, 36.5 years for RM and 37.5 years for RIF. Therefore, the patients from each group were further sub-divided according to female age into younger (36 years or less) and older (37 years or more).

6.2.4.2.1 The incidence of aneuploidy in blastocyst stage embryos generated by a "younger" patient group

Embryos generated by RM and RIF couples where the female partner's age was 36 years or less were analysed. Details about the patients and their embryos included in this younger age group are shown in Table 6-12.

The total aneuploidy rate scored for both patient groups (RM and RIF) was 46.6% (135/290) for average female age 33.4 years (age range 28-36 years). The aneuploidy rate in the RM group was 46.4% (83/179) for average female age 33.5 years (age range 30-36 years) while the aneuploidy rate in the RIF group was 46.8% (52/111) for average female age 33.9 years (age range 28-36 years). No statistical significance was observed in the aneuploidy rate between RM and RIF groups (Fisher's exact test, P = 1.00).

Table 6-12: Details of the "younger" patient group

	RM	**RIF**
Number of patients	21	16
Average maternal age	33.6 years	33.9 years
Number of embryos	179	111

The total number of errors scored in the blastocyst stage embryos from the younger patients was 204 errors (132 errors in the RM group and 72 errors in the RIF group). The ratio of monosomy:trisomy was 1:1 for the blastocysts generated by the RM group. Surprisingly, this ratio was skewed in favour of trisomies in the RIF group (0.6:1), but not statistically significant (Fisher's exact test, P = 0.17). Abnormalities affecting parts of chromosomes were also scored, and accounted for 12.9% and 11% of all errors scored in the RM and RIF groups, respectively.

Abnormal embryos were also grouped according to the number of chromosome abnormalities they were carrying (Table 6-13). The proportion of aneuploid embryos with 1 error was similar for the RM and RIF group (33% and 36%, respectively). The proportion of embryos with 2 errors was almost double in the RM group compared to the RIF group (10% and 4.5%, respectively) and the opposite was found for embryos with 3 or more errors (3.4% and 6.3%, respectively). Although these differences were apparenty quite large, the current study is insufficiently powered to confirm a difference statistically and more samples will need to be collected to verify the significance of this result.

Table 6-13: Frequency of normal and abnormal embryos from younger RM and RIF patients showing no differences in aneuploidy rate between the two groups

PGS indication	**Normal**	**Abnormal**		
		1 error	**2 errors**	**3 or more**
RM	96 (53.6%)	59 (33%)	18 (10 %)	6 (3.4%)
RIF	59 (53.2%)	40 (36%)	5 (4.5%)	7 (6.3%)

6.2.4.2.2 The incidence of aneuploidy in blastocyst stage embryos generated by an "older" patient group

Embryos and patients from all groups (RIF, RM and AMA) were included, and their details are shown in Table 6-14. The aneuploidy rate in the AMA group was 63.8% (294/461) [average female age 40.6 years, age range 37-47 years], in the RM group the aneuploidy rate was 64.3% (72/112) [average female age 40.3 years, age range 37-43 years] while in the RIF group the aneuploidy rate was 60.3% (85/141) [average female age 39.8 years, age range 37-44 years). No statistical significance was observed in the aneuploidy rate between AMA and RM, AMA and RIF or RM and RIF groups.

Table 6-14: Details of the older patient group

	AMA	**RM**	**RIF**
Number of patients	99	20	26
Average maternal age	40.6 years	40.3 years	39.8 years
Number of embryos	461	112	141

The total number of errors scored in the blastocyst stage embryos from the older patients was 812 errors (549 errors in the AMA group, 130 errors in the RM group and 133 errors in the RIF group).

No statistical significance was observed between chromosome gains and losses, as the ratio monosomy:trisomy was almost 1:1 for the AMA and the RM groups (1.17:1 for AMA and 1.06:1 for RM). However, the ratio showed some apparent deviation in RIF patients, in favour of trisomies (0.87:1) (non- significant; Fisher's exact test, P = 0.6). Partial abnormalities accounted for 6.3%, 11.5% and 7.7% of errors scored in the AMA, RM and RIF groups, respectively.

The frequency of normal and abnormal embryos (subdivided into those with 1, 2, and 3 or more errors) from this older patient group (AMA, RM and RIF) is presented in Table 6-15. No differences were observed in the number of aneuploidies per embryo in the three patient groups. Embryos with three or more errors appeared less common in the RIF group compared to the AMA and RM groups, but this was not significant (Fisher's exact test, P = 0.1 for both groups).

Table 6-15: Frequency of normal and abnormal embryos from the older patient group. No differences were observed in the number of aneuploidies per embryos in the three groups

PGS Indication	**Normal**	**Abnormal**		
		1 error	**2 errors**	**3 or more**
AMA	167 (36.2%)	152 (33%)	81 (17.6%)	61 (13.2%)
RM	40 (35.7%)	38 (33.9%)	18 (16%)	16 (14.3%)
RIF	56 (39.7%)	48 (34%)	26 (18.4%)	11 (7.8%)

6.2.5 Prevalence of embryos with aneuploidies potentially compatible with implantation

Cleavage and blastocyst stage embryos having a single aneuploidy with the potential to form a clinical pregnancy and/or a live birth, i.e. trisomy 13, 16, 18, 21 and 22 and abnormalities of sex chromosome copy number were examined further and contrasted with embryos affected by single trisomies affecting other chromosomes or single monosomies affecting any chromosome.

6.2.5.1 At the cleavage stage

The proportion of aneuploid embryos potentially capable of forming a clinical pregnancy out of all analysed embryos was compared with those carrying other chromosome abnormalities in each of the three groups of patients (AMA, RM and RIF). Our data showed that 6.1%, 6.2% and 6.9% of embryos from AMA group (26/426), RM group (9/145) and RIF group (9/130) carried abnormalities with a possibility of producing a clinical pregnancy. No significant differences were observed between different patient groups. Similarly, there were no differences in the rates of embryos carrying a single aneuploidy incompatible with a clinical pregnancy [17.6%, 16.6% and 17.7% of embryos from AMA (75/426), RM (24/145) and RIF (23/130) respectively].

6.2.5.2 At the blastocyst stage

Abnormal embryos capable of pregnancy formation represented 8.3%, 9.7% and 11.5% of all analysed embryos generated by the AMA (40/484), RM (28/290) and RIF groups (29/252), respectively. No significant differences were observed between different patient groups. Similarly, there were no differences in the incidence of embryos incompatible with pregnancy formation between the three patien groups: 24.9%, 23.8% and 23.4% of embryos from AMA (119/484), RM (69/290) and RIF (59/252) carried a single error incompatible with implantation and pregnancy.

6.2.6 Implantation and pregnancy rates

6.2.6.1 Outcomes following cleavage stage comprehensive chromosome analysis

To date, 87 patients have completed their cycles. Of these, only 29 (33%) couples generated at least 1 euploid embryo which was subsequently transferred, whereas the remaining 58 (67%) had only aneuploid embryos, and for this reason did not receive a transfer. The average female age of women who underwent transfers was 38 years (age range 30-44 years). A total of 65 cleavage stage embryos were classified as normal and 45 were transferred to the uterus on day 5 post insemination. Seventeen embryos implanted in fourteen different patients leading to ongoing pregnancies. One of these patients became pregnant with monozygotic twins after the transfer of one embryo, three were pregnant with twins after transferring two embryos, and there were also ten singleton pregnancies. Therefore, the pregnancy rate per transfer seen for patients regardless of PGS indication was 48.3% and the embryo implantation rate was 37.8%. Pregnancy rate per cycle was lower at 16% and this was mainly due to the high number of cycles that had only abnormal embryos (67%). A breakdown of the number of completed cycles, patients who achieved pregnancies and those who did not according to PGS indication, i.e. AMA, RM and RIF is shown in Table 6-16.

75% of AMA patients, 50% of RM patients and approximately 54% of RIF patients had no normal embryos to be transferred.

Table 6-16: Implantation and pregnancy rates for embryos generated from the AMA, RM, and RIF patient populations. AMA group had a very low pregnancy rate per cycle due to the very high number of cases with no normal embyros

Clinical outcome details	AMA	RM	RIF	Total
Number of completed cycles	58	16	13	87
Number of patients who achieved a pregnancy	5	6	3	14
Number of patients who did not achieve a pregnancy	10	2	3	15
Number of cases with no transfer (no normal embryos)	43	8	7	58
Total number of embryos transferred	20	14	11	45
Total number of embryos transferred which implanted	5	8	4	17
Pregnancy rate per cycle	8.6%	37.5%	23%	16.1%
Pregnancy rate per transfer	33.3%	75%	50%	48.3%
Implantation rate per transfer	25%	57.1%	36.4%	37.8%

The implantation and pregnancy rates were also calculated for the younger and older patient groups in order to investigate the effect of age. Details about these clinical outcomes are outlined in Table 6-17. The pregnancy rate per cycle decreased for “older” compared to the “younger” group [60% to 27% for the RM group and 40% to 13% for the RIF group] as a result of increased aneuploidy rate with advanced female age and therefore more cycles did not have any normal embryos suitable for transfer. However, this decline was not significant (Fisher’s exact test, $P = 0.3$ and 0.5, respectively) probably as a result of the small number of cases and therefore it was not possible to draw any firm conclusions. Pregnancy and implantation rates per transfer were very similar for the “younger” RM and RIF groups whereas for the “older” group, pregnancy rate per transfer was similar for AMA and RIF groups (33%) but very high for the RM group (100%). Moreover, the implantation rate per transfer was 25% for the AMA group, 40% for the RIF and 83% for the RM group.

Table 6-17: Clinical outcomes of younger and older RIF and RM patients (cleavage stage)

Clinical outcome details	27-36 years		37 or more years	
	RM	RIF	RM	RIF
Number of completed cycles	5	5	11	8
Number of patient who achieved a pregnancy	3	2	3	1
Number of patient who did not achieve a pregnancy	2	1	0	2
Number of cases with no transfer (no normal embryos)	0	2	8	5
Total number of embryos transferred	8	6	6	5
Total number of embryos transferred which implanted	3	2	5	2
Pregnancy rate per cycle	60%	40%	27.3%	12.5%
Pregnancy rate per transfer	60%	66.7%	100%	33.3%
Implantation rate per transfer	37.5%	33.3%	83.3%	40%

Additionally, the observed miscarriage rate of the completed cases was calculated at 16.7% (1/6) for the RM group, and no miscarriages were observed for the AMA and RIF group. The predicted probability of achieving a pregnancy and maintaining it was calculated according to Brigham's formula at 68.6% and the miscarriage rate at 31.4%. The predicted miscarriage rate was almost double the observed miscarriage rate and this decline in the actual calculated miscarriage rate in the studied group was significant (Fisher's exact test, P = 0.03).

6.2.6.2 Following blastocyst stage comprehensive chromosome analysis

One hundred and twenty one patients who underwent TE biopsy have completed their treatment cycles; of these, 79 (65.3 %) generated at least one euploid embryo and underwent a transfer, whereas the remaining 42 (34.7%) had only chromosomally abnormal embryos, and therefore did not have a transfer.

The average female age of the patients who had a normal embryo was 39.3 years (age range 29-46 years). Of the 215 (47%, 215/457) euploid blastocysts, only 148 were transferred as the guideline in the UK recommends for up to 2 embryos to be transferred in a cycle. Two cases which were performed in USA had 3 embryos transferred. The remaining euploid blastocysts were frozen. Of these, 17 (11.5%, 17/148) were transferred on day-6 of development, and 131 (88.5%, 131/148) were vitrified, and transferred in a subsequent cycle.

Eighty six embryos from sixty three different couples implanted leading to ongoing pregnancies and deliveries. Among these patients, three became pregnant with triplets, 19 were pregnant with twins, and 41 had singleton pregnancies. Thus, the pregnancy rate per transfer regardless of PGS indication was 82.3% and the implantation rate was 57.3%. The pregnancy rate per cycle was lower at 53.7% and this decrease was due to the number of cycles that only had abnormal embryos (42/121). A breakdown of numbers of completed cycles, patients who achieved pregnancies and those who did not after transferring normal embryos according to PGS indication, i.e. AMA, RM and RIF is shown in Table 6-18.

Amongst patients undergoing PGS due to AMA who completed their treatment, 42% did not receive a transfer after generating only abnormal embryos, while a significantly lower proportion of patients (24.6%) who had PGS for RM or RIF did not receive a transfer (Fisher's exact test, $P = 0.035$).

Additionally, the miscarriage rate was calculated at 4% (1/22) for the AMA group, 18% for the RM group and 6% from the RIF group. The predicted pregnancy rate according to Brigham's formula for patients of the RM group who achieved a pregnancy is calculated at 72.8% and therefore the predicted miscarriage rate is 27.2%, although apparently higher than the observed miscarriage rate following PGS, this did nt reach statistical significance (Fisher's exact test, $P = 0.17$).

Table 6-18: Implantation and pregnancy rates for blastocysts generated from the AMA, RM, and RIF patient populations

Clinical outcome details	AMA	RM	RIF	Total
Number of completed cycles	64	29	28	121
Number of patient who achieved a pregnancy	26	22	17	65
Number of patient who did not achieve a pregnancy	10	1	3	14
Number of cases with no transfer (no normal embryos)	28	6	8	42
Total number of embryos transferred	68	39	43	150
Total number of embryos transferred which implanted	36	27	23	86
Pregnancy rate per cycle	40.6%	76%	61%	53.7%
Pregnancy rate per transfer	72%	96%	85%	82%
Implantation rate per transfer	53%	69%	53%	57%

In order to investigate the effect of female age on implantation and pregnancy rates, completed cycles from younger and older patient groups who had their embryos screened at the blastocyst stage were analysed. These results are presented in details in Table 6-19 for patients with RM and RIF.

Table 6-19: Clinical outcomes of younger and older RIF and RM patients (blastocyst stage)

Clinical outcome details	27-36 years		37 or more years	
	RM	RIF	RM	RIF
Number of completed cycles	12	9	17	19
Number of patient who achieved a pregnancy	12	6	10	11
Number of patient who did not achieve a pregnancy	0	1	1	2
Number of cases with no transfer (no normal embryos)	0	2	6	6
Total number of embryos transferred	21	16	18	27
Total number of embryos transferred which implanted	14	8	13	15
Pregnancy rate per cycle	100%	67%	59%	58%
Pregnancy rate per transfer	100%	86%	91%	85%
Implantation rate per transfer	67%	50%	72%	56%

It was evident from the analysis of this data that the pregnancy rate per cycle decreased significantly with advancing maternal age for the RM patients (100% for younger vs. 59% for the older patients) (Fisher's exact test, P = 0.02). There was also a decline for RIF patients (67% for younger vs. 58% for the older patients) but this was not statistically significant (Fisher's exact test, P = 1.00). This decrease in pregnancy rate is accompanied by a tendency for the older patient groups to generate larger numbers of aneuploid embryos, and therefore have fewer transfers. The pregnancy rate per cycle for advanced female age group was 41%, which was not significantly different from that observed for "older" patients with RM or RIF (59% and 58%, respectively). Additionally, no significant difference was observed between the pregnancy rate per transfer or the implantation rate between younger and older RM and RIF patients (no decrease in the implantation rate with age when normal embryos were transferred as can be seen in Table 6-19), but this might be attributable to the small number of cases with known outcome in each group.

We were also interested in examining the possible difference in outcomes after transferring blastocysts on day-6 or vitrifying and transferring in a subsequent "natural" cycle (Table 6-20). No significant variability was observed between fresh and frozen transfers either for the pregnancy rate, which was 77% and 83% respectively, or the implantation rate (57% and 61% respectively).

Table 6-20: Pregnancy and implantation rates after fresh embryo transfer and vitrified/thawed transfers showing no differences in the pregnancy and implantation rate per transfer between the two groups

Clinical outcome details	Fresh day 6 transfer	Vitrified/thawed transfer
Number of completed cycles	37	84
Number of patient who achieved a pregnancy	10	55
Number of patient who did not achieve a pregnancy	3	11
Number of cases with no transfer (no normal embryos)	24	18
Total number of embryos transferred	18	131
Total number of embryos transferred which implanted	11	75
Pregnancy rate per cycle*	27%	65%
Pregnancy rate per transfer*	77%	83%
Implantation rate per transfer	61%	57%

*Pregnancy rates per cycle and per transfer cannot be reliably compared between those two groups as most of the vitrified cycles were performed in USA and embryo transfer strategies are different to that in the UK (3 versus 2 embryos can be transferred)

6.3 Summary of findings

A significant decrease in aneuploidy rate was observed between the cleavage and blastocyst stage for patients undergoing PGS due to AMA, RM or RIF. Moreover, a significant decrease in aneuploidy rate was found between RM patients and AMA and RIF groups. Frequency of chromosome aneuploidy was similar in embryos from all three groups apart from abnormalities affecting chromosomes 2 and 22, which were significantly more common amongst embryos of the RM group.

Additionally, the miscarriage rate for RM patients who underwent PGS at the cleavage stage was significantly lower than that predicted according to Brigham's formula. Recurrent miscarriage patients who had PGS at the blastocyst stage also saw an apparent improvement in the probability of maintaining a pregnancy, although this did not reach statistical significance.

No adverse affects of embryo cryopreservation were detected, in terms of implantation or pregnancy rates per transfer.

7. Discussion

7.1 A detailed cytogenetic investigation of embryos at the final stage of development before implantation

While numerous studies have been performed to assess the cytogenetics of human cleavage stage embryos, very few investigations have examined the blastocyst stage. A small number of previous studies have explored the chromosome constitution of human blastocysts using FISH, a method which permits testing of multiple cells from each embryo, but only allows the screening of a limited number of chromosomes (up to 12) (Gonzalez-Merino et al., 2003; Coonen et al, 2004; Daphnis et al, 2005, 2008; Santos et al., 2010). Other studies have used conventional cytogenetic banding methods or metaphase CGH or aCGH. These techniques screen the entire chromosome compliment (24 chromosomes), but technical and/or financial considerations mean that results are usually obtained from only one cell (or one biopsy specimen composed of a small number of cells) (Clouston et al., 2002; Fragouli et al., 2008, 2010; Traversa et al, 2011). In the current study it was decided to combine FISH, metaphase CGH and aCGH, in an attempt to provide a more detailed insight into the chromosomes of embryos immediately prior to implantation. It was hoped that employing comprehensive aneuploidy screening methods (i.e. CGH and aCGH) would reveal the abnormality rate for individual chromosomes, while utilisation of a technique applicable to large numbers of cells from each embryo (i.e. FISH) would provide information about mosaicism rates.

At the time that these experiments were initiated, the 24Sure platform (BlueGnome Ltd), a BAC microarray specifically designed for the cytogenetic analysis of human embryos, was a new innovation with no peer-reviewed validation data concerning its accuracy. Consequently, it was also considered important to evaluate the reliability of this approach when applied to blastocyst biopsies, equivalent to clinical samples. Although metaphase CGH had been previously validated and employed for PGS and PGD purposes (Fragouli et al., 2008; 2010; Alfarawati et al., 2011a or 2011b), the amount of time it required to yield results was not compatible with a fresh blastocyst transfer. Hence, embryos had to be cryopreserved and transferred in a subsequent unstimulated

cycle. aCGH is an attractive alternative because it is capable of yielding results in 24 hours, and therefore could be combined with a day-5 blastocyst biopsy followed by a day-6 fresh transfer. In the time since the current research was initiated, the 24Sure platform has been validated for the analysis of polar bodies biopsied from human oocytes (Geraedts et al, 2011; Magli et al, 2011) and blastomeres biopsied from cleavage stage embryos (Gutierrez-Mateo et al, 2011). However, the work reported here remains the only assessment of this microarray platform applied to blastocysts.

The current study confirmed a high efficiency for both metaphase CGH and aCGH [98% (51/52) and 100% (20/20) of samples yielded results for CGH and aCGH respectively]. This efficiency was similar to that observed in previous studies involving metaphase CGH carried out by our group (Fragouli et al., 2008, 2010), suggesting that aCGH provides a result at a similar frequency to metaphase CGH. This has subsequently been confirmed in more than 273 blastocyst biopsy samples tested in our laboratory for clinical purposes (94.2% diagnosis rate). Analysis of multiple nuclei from embryos fixed on microscope slides showed high concordance between all methods used. Specifically, metaphase CGH and FISH were in agreement for 94% of the investigated embryos and aCGH and FISH for 100% of embryos. For embryos that had results available from all three cytogenetic methods, metaphase CGH and aCGH were all concordant on 89.5% of occasions. All discrepancies observed were a consequence of mosaicism, confirmed by FISH analysis of the embryos in question.

Of the fifty two analysed blastocysts, 42% were found to be euploid (mean age 36.1 years). Dividing this group of women into two subgroups according to maternal age, an older ($\geq$37 years) and a younger ($\leq$36 years), revealed, as expected, a significant increase in blastocyst aneuploidy rates for the older group (30.4% versus 75.9%; P = 0.002). This increase was similar to the one noted previously in a study where 136 blastocysts were examined (Fragouli et al, 2008).Thus, it is clearly the case that embryos from women of advanced reproductive age are frequently capable of successfully reaching the blastocyst stage, but they are at significantly elevated risk of

being chromosomally abnormal. These observations are also in line with earlier investigations examining oocytes and cleavage stage embryos, where the adverse effect of female age on chromosome segregation was extensively examined (Angell et al., 1983; Delhanty et al., 1993; Munné et al., 1993; Iwarsson et al., 1999; Wilton et al., 2003; Fragouli et al., 2008; 2010; 2011).

The use of FISH provided in an opportunity to estimate which aneuploidies had arisen during meiosis and which has a post-zygotic origin. The logic used to distinguish meiotic from mitotic errors was that abnormalities derived from gametes (meiotic) were likely to present in all examined cells of the embryos, whereas aneuploidies present in some cells and not in others were more likely to be post-zygotic in origin. Of course, it is possible that an abnormality arising in one of the first mitotic divisions following fertilisation, or a trisomy/monosomy rescue event occurring soon after fertilisation, could conceivably result in an embryo in which the vast majority/all cells were of identical karyotype, with the appearance of a meiotic event. However, given that the blastocyst stage is only 5-6 cell generations (doublings) from the first mitotic division, there is not much opportunity for one cell line to be entirely overgrown or eliminated, and consequently the risk of such interpretation seems low.

Almost a third of the examined embryos were affected by abnormalities of a predicted meiotic origin. Of these, 25% only carried abnormalities of meiotic origin, with the remainder consisted of a combination of meiotic and post-zygotic errors. The vast majority (11/13) of the embryos which were classified as aneuploid solely due to meiotic errors were carriers of a single chromosome abnormality. A recent study by Santos et al. (2010) using FISH to examine 10 chromosomes (1, 7, 13, 15, 16, 18, 21, 22, X and Y) in 50 embryos at the blastocyst stage of preimplantation development also found that meiotic errors were common, observed in 14% of all analysed embryos. However, meiotic errors were almost absent in two previous studies where FISH for 3 chromosomes (18, X and Y) was also employed to examine blastocysts (Coonen et al., 2004; Daphnis et al., 2005). This difference in the incidence of meiotic aneuploidy between the current

study and previous FISH-based studies is most likely attributed to the use of different cytogenetic methodology and the lower average female age (36 years in this study compared to 33 years for both other studies). As FISH only allows examination of a small number of chromosomes, it is inevitable that some uniformly aneuploid embryos will not be detected and will be wrongly characterised as euploid (Coonen et al., 2004; Daphnis et al., 2005).

A third of all tested blastocysts were found to carry varying degrees of mosaicism. Chromosome abnormalities were observed in every cell analysed in approximately half of the mosaic embryos (15% of all tested embryos). One of the mosaic aneuploid blastocysts tested was characterised as 'chaotic' due to the presence of multiple cytogenetically distinct aneuploid cell lines. The other mosaic blastocysts (17% of all tested embryos) were seen to have both a diploid and an aneuploid cell line and were therefore classified as mosaic diploid-aneuploid. As discussed below, it is this final group of embryos that represent the greatest risk in terms of clinical misdiagnosis. Overall, the results are similar to previous, FISH-based studies, which recorded mosaicism at the blastocyst stage. Both Santos et al (2010) and Coonen et al. (2004) concluded that on day-5 the rate of mosaicism was approximately 55%.

Previous comprehensive chromosome analysis studies performed at the cleavage stage have shown that only a quarter of examined embryos were uniformly diploid and three quarters were either uniformly aneuploid or mosaic (Voullaire et al, 2000; Wells and Delhanty, 2000). Moreover, Vanneste et al (2009) examined all blastomeres of 23 cleavage stage embryos and reported a mosaicism rate of 87% (8 embryos were mosaic diploid-aneuploid and 12 embryos were mosaic aneuploid). These results in combination with data obtained from the above mentioned studies suggest that the levels of mosaicism peak at the cleavage stage of preimplantation development, but some degree of resolution occurs during progression to the blastocyst stage. The decline in mosaicism rates is likely attributed to two factors: at the level of the embryo, the death of those that have experienced a complete breakdown in mitotic chromosome segregation (cha-

otic embryos) prior to ICM and TE differentiation; at the level of the cell the loss of those that are highly abnormal, perhaps via some form of programmed cell death. It is conceivable that blastomeres with compromised cell cycle machinery, predisposing to chaotic chromosome segregation, are tolerated at the cleavage stage, as the embryonic genome is not activate during the first few mitotic divisions (Braude et al., 1988). Once the genome is activated, it is likely that cell cycle checkpoints and other regulatory mechanisms would recognise highly abnormal cells/embryos and eliminate them.

Some concerns regarding the ability of comprehensive chromosome analysis techniques, such as metaphase CGH and aCGH, to accurately diagnose mosaic blastocyst embryos have been raised. However, the results of this study show that approximately half of all mosaic embryos did not consist of any normal cells and would therefore be classified as abnormal regardless of which cells were biopsied. Mosaic diploid aneuploid embryos could, however, be more problematic. In the current investigation, such embryos accounted for 17% of examined blastocysts. These findings are in agreement with results from a previous study where 90 blastocysts where examined with three-colour FISH and 17% were found to be mosaic diploid-aneuploid (Bielanska et al 2005). The proportion of diploid-aneuploid mosaicism at the blastocyst stage is lower than that reported at the cleavage stage (34%, Vanneste et al, 2009). The ultimate fate of such embryos, and whether any have the capacity to form viable pregnancies, is not known. If, as speculated above, aneuploid cells are at a growth disadvantage relative to chromosomally normal cells or are eliminated through apoptosis, an apparently normal embryo might ultimately emerge, with the potential for forming a viable pregnancy. Support for this hypothesis can be obtained from the study of Santos and colleagues (2010) who not only observed a steady decrease in the mosaicism rate from 56% on day-5 to 42% on day-8, but also found a positive correlation between the total number of cells and the proportion of euploid cells per embryo in day-5 and day-8 embryos. Therefore, there is evidence that some blastocysts with the capacity for producing a normal foetus but might be incorrectly discarded due to mosaicism. However, the risk of such a misdiagnosis probably only ap-

plies to embryos with a high proportion of euploid cells. Most of the aneuploidies observed at the blastocyst stage during this study are not compatible with pregnancy formation and it seems reasonable to speculate that embryos in which the majority of cells carry aneuploidies of this type are likely to suffer from impaired development. From the data presented in this thesis it is evident that only 6% of all examined blastocysts were mosaic diploid aneuploid and consisted of more than 50% normal cells.

Preimplantation screening of embryos at the blastocyst stage is based on a biopsy of TE cells. Given the existence of confined placental mosaicism in some chorionic villus samples (a tissue derived from the trophectoderm lineage), the question of whether the TE provides a truly representative sample of the rest of the embryo has been raised. Previous studies have suggested that there is no preferential allocation of abnormal cells to the trophectoderm layer and have provided reassurance that it provides an accurate representation of the entire embryo (Evsikov and Verlinsky, 1998; Magli et al., 2000; Fragouli et al., 2008; Northrop et al., 2010). Furthermore, a recent study by Mamas et al (2012) used a mixture of normal and abnormal cells in order to mimic a mosaic TE biopsy and it was confirmed that aneuploidy can be confidently detected when the proportion of aneuploid cells was ≥50%.

Comprehensive chromosome analysis of human embryos at the blastocyst stage has shown that aneuploidy affects all chromosomes and that both monosomies and trisomies can survive to the final stage of preimplantation development *in vitro* (Fragouli et al, 2008; Northrop et al, 2010). The results presented here agree with these previously published findings. Chromosomes 22, 16, 15, 21 and X were most frequently found to be abnormal, types of aneuploidy often seen in tissue from miscarriages. However, almost all chromosomes were seen to participate in aneuploidy events in blastocysts, including chromosomes never seen to be abnormal in prenatal samples or miscarriages, underscoring that many aneuploidies incompatible with formation of a clinical pregnancy are tolerated at this early stage. The number of abnormalities of a post-

zygotic origin was double that of meiotic origin (40 versus 19, respectively) and mosaicism rates were lower than typically seen at the cleavage stage. Together, this data highlights the extreme chromosomal instability of the cleavage stage, with multiple mitotic errors occurring in early divisions, presumably followed by elimination of the most severely affected cell/embryos.

Of all scored errors, 26 were chromosome gains (CG, 10 meiotic and 16 post-zygotic), 22 were chromosome losses (CL, 9 meiotic and 13 post-zygotic) and 11 were the result of mitotic non-disjunction (MND, reciprocal losses and gains in daughter cells). This data is in contrast with findings reported by Coonen et al. (2004) and Daphnis et al. (2005) where 299 and 42 blastocysts were analysed, respectively. Both these studies suggested that anaphase lag leading to CL was the most frequently observed aneuploidy causing mechanism, whereas the results presented here show that CGs and CLs were occurring in an equal manner. This could be attributed to the fact that two different molecular cytogenetic methods have been employed, and also to the difference in the number of analysed embryos. It is known that FISH analysis of single cells tends to over-estimate chromosome loss (e.g. monosomy) due to problems including probe hybridisation failure and overlapping signals (Colls et al, 2007).

To conclude, data obtained in this part of the project provide a validation of the aCGH for the screening of blastocyst stage embryos for aneuploidy. The results concerning the incidence of mosiaicism suggest that only a small proportion of potentially viable blastocysts (~6%) are at risk of misdiagnosis. Therefore, embryo screening at the blastocyst stage using CGH or aCGH appears to be an accurate tool to distinguish chromosomally normal from chromosomally abnormal blastocysts, potentially assisting in the identification of viable embryos with higher chances to implant. This is a particularly important consideration for assisted reproductive cycles involving single embryo transfer (SET).

7.2 The effect of aneuploidy on embryo morphology and development to the cleavage and blastocyst stage

Assessment of embryo morphology is the main method used in IVF clinics to choose the most appropriate embryos to be transferred to the uterus. Previous studies have examined the associations between aneuploidy and several distinct abnormal morphologies at the cleavage stage, such as multinucleation, fragmentation, unevenness of mitotic divisions, and developmental rate (Munné et al, 1995c; Staessen and Van Sterteighem, 1998; Marquez et al, 2000; Hardarson et al, 2001, 2003; Magli et al, 2001; Moayeri et al, 2008). Most of these studies reported no or a weak correlation. No morphological marker capable of reliably differentiating between normal and aneuploid embryos has yet been identified.

This failure may be a consequence of biological or technical factors. It may simply be the case that aneuploidy has little biological impact at the cleavage stage and consequently has no effect on morphology. Alternatively, the methods of identifying aneuploidy used in previous studies may have failed to accurately reveal chromosomally abnormal embryos (e.g. the incomplete chromosomal analysis provided by FISH), limiting attempts to assess the impact of aneuploidy on morphology. This study sought to address some of the difficulties of previous studies, applying comprehensive chromosome screening (aCGH and metaphase CGH) to investigate possible relationships between embryo morphology at the cleavage and blastocyst stages.

The aneuploidy rate for the examined cleavage stage embryos was 83.5% (323/367). Amongst grades which are typically chosen for transfer (i.e. grade 1, 2 or 3), no difference in morphology was observed between normal and abnormal embryos. Specifically, 30% of normal embryos were grade 1 (6-8 blastomeres with no fragmentation or multinucleation), similar to the proportion of abnormal embryos of this same grade (23%). These results are in agreement with those described in other studies that examined the cytogenetics of the cleavage stage with the use of either metaphase or aCGH (Voullaire et al, 2000; Wells and Delhanty, 2000; Gutierrez-

Mateo, 2011). Conversely, a significant overrepresentation of abnormal embryos was observed for embryos with ≥ 20% fragmentation or with very uneven cell size (grade 4) (1.6% of euploid vs. 9.6% of aneuploid embryos) (P = 0.04). These findings are concordant with previous studies where the fragmentation rate was compared with aneuploidy and a weak positive correlation was found between the two (Magli et al, 2001; Magli et al, 2007; Moayeri et al, 2008).

At the blastocyst stage more than half (56.5%) of all embryos investigated were chromosomally abnormal. As far as the effect of aneuploidy on blastocyst morphology was concerned, a general increase in the frequency of abnormalities among embryos with poor morphologic scores (≤ 3BB) was observed, along with a greater likelihood of euploidy for embryos with good scores (≥ 5BB). However, as with the cleavage stage, the relationship between blastocyst morphology and chromosome status was relatively weak. Specifically, half (51%) of embryos achieving the highest degree of development on day-5 (grades 5 and 6) were euploid, compared to 32% of embryos graded <3. A recent randomized clinical trial performed by Yang et al (2012), also provides data suggesting that embryos affected by lethal chromosome anomalies are often inadvertently transferred to patients when they are chosen based upon morphological criteria. In the trial, clinical outcome parameters (implantation and pregnancy rates) were compared between a control patient group of good prognosis younger women who underwent a day-6 SET, with embryo selection based upon morphology, and a similar test patient group who also had day-6 SET, but with embryo selection dependent on the results of PGS via aCGH. It was evident from the obtained results that an approach involving morphological and cytogenetic assessment, along with blastocyst transfer significantly improved clinical outcomes (ongoing pregnancy rate for control group: ~42%, and for test group: ~70%, P=0.009).

In theory, for the patients examined in this part of the project, the risk of transferring an aneuploid embryo could have been reduced from 83% at the cleavage stage and 56% at the blastocyst stage (if no morphologic analysis was performed) to 80% and 48% if only embryos of Grade

1 at the cleavage stage and of grades 5/6 at the blastocyst stage were transferred. However, in order to achieve this modest reduction in aneuploidy risk it is necessary for patients to produce one or more embryos of the highest morphological quality, which is often not the case during IVF cycles.

Not surprisingly, a strong association was observed between maternal age and aneuploidy at the cleavage and the blastocyst stages, an observation in agreement with a large quantity of previously published data (Angell et al., 1983; Delhanty et al., 1993; Munné et al., 1993; Iwarsson et al., 1999; Wilton et al., 2003; Fragouli et al., 2008; 2010; 2011).

The effect of specific types of chromosome errors on cleavage and blastocyst stage morphology was also assessed. An interesting finding was that the frequency of aneuploid embryos carrying a single monosomy (when considering only those embryos with a single aneuploid chromosome) decreased as development progressed from the cleavage to the blastocyst stage (51.5% of day-3 embryos, 43.8% of day-5 embryos) while aneuploid embryos affected by a single trisomy increased from the cleavage to the blastocyst stage (36.1% of day 3 embryos, 48.6% of say 5 embryos). This decline is probably reflective of the lethality of this type of abnormality. For embryos affected by a single aneuploidy at the cleavage stage, the ratio of monosomy to trisomy in good quality (grade 1) embryos was 2.7:1 which reflected a statistically significant overrepresentation of monosomies ($P = 0.04$), whereas the opposite was seen in poor quality (grade 4) embryos, where the ratio was 1:1.5. Moreover, at the blastocyst stage the ratio of monosomy to trisomy in fast-growing (grade 5 or 6) embryos with a single aneuploidy was 1:1.7, whereas slower-growing blastocysts (grade ≤3) an almost equal frequency of monosomies to trisomies was observed (1.1:1).

As far as the incidence of complex (3 or more) errors was concerned, it did not seem to affect morphology at the cleavage stage, as it was observed in almost equal frequency for all 4 grades. Blastocysts with complex chromosome errors were also seen, but unlike the cleavage

stage, these embryos were a small minority (declined significantly from 40% of cleavage to 10% of blastocyst stage embryos, Chi-squared test, $P < 0.0001$), tended to have poorer TE and ICM, and tended not to achieve full blastocoel expansion as rapidly. These results, along with the fact that multiple chromosome abnormalities have been reported in less than 1% of spontaneous miscarriages, confirms their adverse impact on preimplantation development, but this effect only begins around the time of the first cellular differentiation into TE and ICM.

Interestingly, abnormal embryos with a single potentially viable aneuploidy (e.g. trisomy 13, 16, 18, 21 and 22 and sex chromosome aneuploidies) had morphologies indistinguishable from normal embryos both at the cleavage and the blastocyst stages and did not seem to be selected against during preimplantation development. This finding is consistent with results from previous studies where products of conception (miscarriages) were examined (Hassold et al., 1980; Menasha et al., 2005). These studies found that conceptions carrying abnormalities affecting small (or relatively gene-poor) chromosomes are often capable of implanting and producing clinical pregnancies, but usually end in miscarriage.

The proportion of normal embryos was compared in cleavage stage embryos according to cell number. No significant differences were observed in aneuploidy rates for slow- (<6 cells) and fast-cleaving (≥ 10 cells) embryos compared to normal cleaving (6-9 cells). This observation confirms data obtained by Moayeri et al (2008), who examined nine chromosomes using FISH from embryos generated by patients with an average female age of 37.7 years, and found that cell number did not predict aneuploidy on day-3. On the other hand, it contradicts results from Magli et al (2007) who have suggested that aneuploidy rate is higher in slow-cleaving embryos and in embryos cleaving faster than normal on day-3 (8 chromosomes examined by FISH, female age not mentioned).

The female to male ratio was 1.4:1 at the cleavage stage and almost 1:1 at the blastocyst stage. At the cleavage stage, 72% of fast-cleaving embryos were females (had ≥ 10 cells). This data contradicts previously published studies where male embryos were shown to cleave faster and have greater number of cells from day 2 to the blastocyst stage (Ray et al, 1995). On the other hand, male embryos achieved slightly accelerated growth rates than equivalent female embryos at the blastocyst stage: 55% of grade 5 and 6 blastocysts were found to be male, whereas 53% of slower growing (grade 3) blastocysts were found to be female. A recent retrospective population-based study which included 13,165 women who had single embryo transfer showed that single blastocyst transfer skews the secondary sex ratio (sex ratio at birth) in favour of male offspring (Dean et al, 2010). Similar results were observed in the study performed by Luna et al. (2007) following embryo transfer at the blastocyst stage with a skewed female to male ratio in favour of male births. The distinction in embryo growth rate between male and female is unlikely to reflect any differences in competence, but suggests that male embryos tend to undergo more rapid mitoses during the latter preimplantation stages and therefore form expanded blastocysts sooner than females. Since degree of blastocoel expansion is generally considered a positive morphological indicator of embryo quality, more advanced blastocysts (which have a greater chance of being male) are more likely to be selected for transfer to the uterus, thus causing a small shift in the sex ratio. Although Weston et al. (2009) reported no differences in the sex ratio following the transfer of 435 blastocysts, the study carried out by Dean and colleagues (2010) is likely to be more reliable as they only looked at single embryo transfers and assessed more than 13,000 cases. It is also possible that other interventions during fertility treatment might affect the sex ratio and cause differences between individual clinics (e.g. use of IVF or ICSI) (Dean et al., 2010).

The underlying biologic cause for the blastocyst male to female sex ratio difference remains unknown at this time. Burgoyne (1993) suggested that the presence of a growth factor gene(s) on the Y-chromosome might have the ability to accelerate the preimplantation development of male embryos. It had also been suggested that differences in gene dosage compensation

related to X-chromosome inactivation during early preimplantation development might hinder female embryos (Van den berg et al, 2009).

In summary, cleavage stage embryos typically chosen for transfer depending on morphological grading (grade 1-3) have no significant differences in the incidence of euploidy and aneuploidy and only embryos with poorest morphology, which are not usually chosen for transfer, have an excess of aneuploidy. Most forms of aneuploidy were shown to be compatible with blastocyst formation, although the greater the number of genes involved (i.e., the larger the chromosome or the more individual chromosomes), the slower the development and the poorer the morphology of the blastocyst tended to be. Blastocyst morphology and aneuploidy are therefore linked, but morphologic analysis cannot be relied on to ensure transfer of chromosomally normal embryos. A significant proportion of aneuploid embryos were capable of achieving the highest morphologic scores, and some euploid embryos were morphologically poor. At the present time, invasive methods of embryo sampling remain the only effective means of detecting aneuploidy at the blastocyst stage.

7.3 Preimplantation Genetic Diagnosis of chromosome rearrangements using comprehensive molecular cytogenetic methodology

During this part of the project a universal diagnosis method was applied to detect chromosome imbalances in human oocytes and embryos. These were generated by carriers of reciprocal or Robertsonian translocation or inversions.

The diagnostic efficiencies of metaphase CGH and aCGH applied for the PGD of patients who are carriers of chromosome rearrangements were very similar, with 91.5 and 93% of samples examined yielding a result. The efficiency reported for the comprehensive chromosome analysis methods used in this project was similar to diagnostic success rates achieved by experienced PGD laboratories using established FISH protocols (92.9–93.5%) (Munné et al., 2000; Lim et al., 2008; Lledo et al., 2010). It was also equivalent to success rates reported for metaphase CGH and aCGH during PGS, suggesting that the methods used have efficiencies appropriate for clinical embryo testing (Fragouli et al, 2008; Schoolcraft et al., 2010; Gutierez-Mateo et al., 2011; Yang et al., 2012).

The sensitivity of metaphase CGH in detecting partial chromosome losses and gains has been reported to be 4-5 Mbp (Piper et al., 1995). However, it decreases to 5-10 Mbp when it is used for the analysis of single cells (Wells and Delhanty, 2000) because whole genome amplification does not amplify all parts of the genome with the same efficiency. The microarray platforms used during this part of the project (24Sure and 24Sure+, Bluegnome Ltd), have a screening resolution between 2 and 10Mbp, depending on where within the genome the aberration is located (around 2 Mbp in sub-telomeric regions). The smallest abnormality detected in this part of the project was a 2.8 Mbp gain of material derived from the satellite region of chromosome 22. This was achieved with the use of the 24Sure+ high-resolution microarray and provides reassurance that this microarray platform is sufficient for the diagnosis of the vast majority of chromosome rearrangements carried by patients referred for PGD.

The optimal stage to perform a biopsy (polar bodies, blastomeres or trophectoderm) followed by PGD is still controversial. Most PGD cases for carriers of chromosome rearrangements are performed at the cleavage stage (ESHRE PGD consortium, 2012). However, diagnosis at the cleavage stage does not only involve the removal of a cell or two from the embryo which might have a detrimental effect on its viability (Cohen et al., 2007) but also chromosome mosaicism is very common at this stage (Voullaire et al, 2000; Wells and Delhanty, 2000; Vanneste et al., 2009). However, trophectoderm biopsy of at the blastocyst stage has been reported to be much less detrimental (McArthur et al., 2008; Schoolcraft et al., 2009) and mosaicism rate is lower compared to the cleavage stage (as observed in this project). The incidence of mosaicism may compromise the accuracy of comprehensive chromosome screening methods, when they are performed for the PGD for chromosome rearrangement carriers. Nonetheless, mosaicism in general affects chromosomes at random and it is statistically unlikely that the chromosomes involved in the rearrangement will be affected in a particular embryo leading to a possible misdiagnosis. A follow-up analysis performed in this study on day-5 (using FISH) of non-transferred embryos diagnosed abnormal on day-3 (using aCGH) revealed many instances of mosaicism, but most importantly no false positive or false negative diagnoses were observed in terms of the rearranged chromosomes and all embryos diagnosed as aneuploid for additional chromosomes were confirmed to be abnormal. However, it seems likely that mosaicism will rarely result in the elimination of a potentially normal embryo. As an alternative to cleavage stage biopsy, analysis of PBs can be undertaken in cases where the female patient carries the chromosome rearrangement. Such tests should not be compromised by mosaicism, since any abnormalities detected in the oocyte should be found in all the cells of any resulting embryo. On the other hand, abnormalities of paternal origin cannot be assessed using this strategy.

The use of comprehensive chromosome analysis will also detect any aneuploidies affecting chromosomes unrelated to the rearrangement, usually resulting in a reduction of the number of embryos which are considered suitable for transfer. Specifically, half of the embryos identified

to be normal or balanced for the chromosomes involved in rearrangements were seen to be carrying errors affecting other chromosomes. The detection of these aneuploid embryos is one of the principal advantages of comprehensive chromosome analysis methods over traditional FISH protocols, since spontaneously arising chromosome abnormalities are a major cause of abortions or the birth of children with congenital abnormalities or mental retardation.

A total of 39 carriers of structural rearrangements (average maternal age 35.7 years) received genetic diagnosis of their embryos in 48 cycles. Up until the time of writing this report, 31 patients had completed their treatment (39 cycles). A total of 15 cycles had normal embryos transferred to the uterus resulting to a pregnancy rate of 40% per transfer (6/15 patients) and of 19.3% per cycle (6/31 patients). The relatively low pregnancy rate per cycle observed was a result of the high proportion of abnormal embryos produced from these patients, leading to an increased number of cycles (24 cycles) having no normal embryos suitable for transfer.

Fischer et al (2010) used FISH for PGD carried out for carriers of chromosome rearrangements (average female age 34 years) and reported a 25% pregnancy rate per cycle and 39% pregnancy rate per transfer. These results are comparable to the findings of the current study. Considering that the number of chromosomes screened by Fischer et al. was limited, it might be expected that their clinical outcomes would be inferior to the current study (in which comprehensive chromosome analysis was employed). However, the average number of embryos available for testing in the study of Fischer and colleagues was 10.2 (2766/272) per cycle compared to only 5.4 (257/48) in the current study. As with PGS, outcomes are closely associated with the number of embryos available for testing. Cycles with few embryos have a high risk that no embryos suitable for transfer will be identified. Another difference between the two studies is that the average female age was ~2 years younger in the investigation carried out by Fischer et al. The proportion of female patients under 35 years of age was significantly higher in their study [61% (166/272) compared to 31% (15/48), Chi-squared test, P = 0.0002] and that of female patients over 40 was

significantly lower [5% (13/272) compared to 15% (7/48), Chi-squared test, P = 0.02] in their study. Moreover, Fiorentino et al. (2011) used aCGH during the PGD of 24 chromosome rearrangement carriers (average female age 37.4 years) and reported a pregnancy rate of 43% per cycle and 70.6% per transfer. This high pregnancy rate observed was a consequence of a high proportion of cycles with normal embryos available for transfer (61%) compared to 31% in our study and a possible reason for this is the higher number of average embryos tested per cycle in their study compared to our study (6.7 versus 5.4) which could be the result of possibly using a more aggressive patient stimulation protocol which leads to a higher number of oocyests and therefore embryos produced leading to a higher number of transfers. The results from Fiorentino and colleagues suggest that the application of comprehensive chromosome analysis to PGD for chromosome rearrangements can yield excellent results for certain groups of patient. However, as the data presented here shows, for some patients, finding any embryos suitable for transfer is a challenge. This has important implications for counselling and the management of patient expectations.

It has been suggested that the presence of a balanced chromosome rearrangement might affect the alignment of other chromosomes on the meiotic spindle and consequently lead to a generalized increase in aneuploidy or what is known as an inter-chromosomal effect (ICE). Some studies have reported an ICE in spermatozoa derived from translocation carriers, in the form of an elevated frequency of disomy affecting the sex-chromosomes (Rousseaux wt al., 1995; Anton et al., 2008; Vozdova et al., 2011). However, there is controversy, as other investigations found no evidence of increased chromosomal malsegregation in gametes from men carrying Robertsonian (Martin, 1988; Syme and Martin; 1992) or reciprocal (Van Hummeln et al., 1997; Blanco et al., 1998) translocations.

Screening chromosomes of the gametes and preimplantation embryos produced by carriers of chromosome rearrangements is especially valuable when attempting to determine the

presence or absence of an ICE since, at early stages of development, elimination of embryos affected by lethal chromosomal anomalies (via developmental arrest, implantation failure or miscarriage) has not yet taken place. Therefore, the initial incidence of chromosome malsegregation can be assessed.

The use of a CGH and aCGH has enabled us to examine the segregation patterns of >10,000 chromosomes in the 257 test samples derived from patients carrying a rearrangement and almost 200,000 chromosomes in a control group. Since the previous results (discussed above) demonstrated that aneuploidy rates are greatly influenced by the stage at which biopsy is undertaken and the age of the mother, a separate control group was created for each patient, consisting of multiple cases where both partners had normal karyotypes. Patients were matched for female age, undergoing treatment in the same clinic at the same time and having PGS using exactly the same method (metaphase CGH or aCGH) and at the same stage of embryonic development (PBs, blastomeres or TE cells).

An increase in the probability of any given chromosome having undergone malsegregation was observed when samples were compared to the control group (0.051 risk of error per chromosome in material from rearrangement carriers versus 0.047 in controls), and although the difference was small (0.4%) it was statistically significant (Chi square test, $P<0.0001$) suggesting the presence of ICE. A more detailed assessment, considering each of the different classes of rearrangement separately (reciprocal translocation, Robertsonian translocation or inversion), was also undertaken and showed that not all were consistently associated with elevated chromosome malsegregation.

The analysis of data from reciprocal translocation carriers in this study (n=115 samples), compared to the appropriate control group, revealed no difference in aneuploidy rate ($P=0.87$). Regardless of whether polar bodies, cleavage stage embryos or blastocyst stage embryos were examined, no evidence for an ICE was found. This is in agreement with previous reports that sug-

gested the presence of a balanced reciprocal translocation does not affect the segregation of chromosomes not involved in the rearrangement (Van Hummeln et al., 1997; Blanco et al., 1998; Gianaroli et al., 2002). However, some other studies have presented contradictory findings (Anton et al., 2008; Vozdova et al., 2011). It has to be mentioned that in all studies which support or contradict the findings, FISH was the used method and analysis was performed on sperm or day-3 embryos. Comprehensive chromosome analysis and testing of oocytes and blastocysts had not previously been undertaken in this context.

Similar to findings in samples from reciprocal translocation carriers, no ICE was apparent in samples from inversion carriers (P=0.18). However, the number of samples was considered insufficient to conclusively determine whether or not an ICE exists in patients with this type of rearrangement (n=12 samples). The possibility that inversions are associated with an ICE remains unproven at present and requires further exploration. Interestingly, in the current study, one patient with unusually high levels of chromosome abnormality had an inversion in combination with a reciprocal translocation. Whether or not the presence of two rearrangements can, together, produce a potent ICE cannot be determined from a single patient, but remains an intriguing possibility.

Comparison of samples from Robertsonian translocation carriers (n=122) with corresponding control groups revealed a highly significant increase in aneuploidy rate (P=0.0002) indicative of a pronounced ICE. However, the stage of development assessed was found to have a profound influence on the risk of chromosomal malsegregation. Subdivision of the data from carriers of Robertsonian translocations revealed that an ICE was only detectable at the cleavage stage (P<0.0001). No ICE was detected in polar bodies (i.e. oocytes) or samples from blastocysts (P=0.46 and P=0.25 respectively). This unexpected finding suggests that the ICE detected in material from Robertsonian translocation carriers is predominantly mitotic in nature, rather than meiotic. The possibility of an ICE in female or male meiosis cannot be entirely ruled out, but the data

indicates that any chromosome malsegregation due to such a phenomenon is extremely rare and dwarfed by the mitotic ICE. The detection of an ICE in cleavage stage embryos from carriers of a Robertsonian translocation is in agreement with a previous study, although at the time a meiotic origin for the increased aneuploidy had been assumed (Gianaroli et al., 2002).

The data obtained during this study provides strong evidence that the detected ICE is of mitotic rather than meiotic origin, occurring after fertilisation. Restriction of the ICE to the first few mitotic divisions following fertilization has not previously been considered, but may be the explanation for many of the discordant findings reported in the literature concerning an ICE, since previous studies have tended to be entirely focus on either gametes or embryos, not both.

The possibility that early embryogenesis might be particularly sensitive to an ICE is not without precedent. It is well known that human embryos display, during the first few mitotic divisions following fertilization, an elevated frequency of chromosomal malsegregation which frequently results in high degree of chromosomal mosaicism and aneuploidy in cleavage stage embryos (Delhanty et al., 1997; Munné et al., 2002; Katz-Jaffe et al., 2004; Gutierez-Mateo et al., 2011). Genetic instability in early preimplantation embryos extends beyond whole chromosome loss or gain, leading to chromosome breakage and consequent segmental abnormalities in some cases (Wells and Delhanty, 2000; Voullaire et al., 2000; Vanneste et al., 2009). The embryonic genome is, for the most part, inactive until the 4-8 cell stage and a deficiency in cell-regulatory mechanisms, including the cell cycle checkpoints that usually act to maintain chromosome segregation and genomic integrity, may be the underlying cause of the observed instability at this time.

Even though this early developmental phase, characterised by genetic instability, may provide the necessary environment for errors caused by an ICE to be propagated, it does not provide an explanation to how the structurally abnormal chromosome disrupts the normal mitotic process. Several studies looking at human gametes (sperm) from male carriers of chromosome rearrangements, have considered the mechanisms that might give rise to an ICE during meiotic

divisions (Anton et al, 2008, 2010) and provided an insight into why certain segregation modes are favoured in patients with different types of rearrangement, but the reason why some male translocation carriers display an ICE in their sperm and others do not remains obscure.

Similarly, the mechanism by which chromosome rearrangement could disrupt mitosis is not clear. Mitotic recombination is a rare phenomenon generally but in the cells of human embryos, there is a good reason to believe that it occurs at an appreciable frequency as it acts as a mechanism of DNA repair and is usually initiated by a double strand break (DSB). It is becoming increasingly clear that DSBs are very common during early embryonic development and leads to a high frequency of chromosome breakage (Wells and Delhanty, 2000; Voullaire et al., 2000; Vanneste et al., 2009). The detection of micronuclei, which are often seen in the cells of preimplantation embryos and are suggestive of the presence of fragmented chromosomes, provides another evidence of the elevated incidence of DSBs.

One possibility is that mitotic recombination involving rearranged chromosomes, holds together chromosomes that would usually be in separate locations, and may lead to disruption of the arranged chromosomes on the spindle. This is similar to the principle hypothesis concerning a meiotic ICE, in which the pairing of rearranged chromosomes with their structurally normal homologues disrupts the positioning, pairing and segregation of other chromosomes during meiosis (Lejeune et al. 1963; Guichaoua et al., 1990).

A second possibility is the alteration of normal chromosome positioning with the interphase nucleus due to the presence of rearranged chromosomes, which affects the attachment to the mitotic spindle during metaphase. The fact that the ICE detected during this study was only obvious for Robertsonian translocation carriers may be of particular relevance to hypotheses involving chromosome positioning in the nucleus or on the spindle. Robertsonian translocations involve acrocentric chromosomes (chromosomes of D and G group), where the short arms composed of tandem copies of ribosomal RNA (rRNA) genes. These regions co-localise during inter-

phase, acting as a focus for formation of the nucleolus. A Robertsonian translocation involves fusion of long arms of these chromosomes at the centromere, accompanied by loss of the short arms which might lead to the affected chromosomes failing to associate with the nucleolus. Since most chromosomes have specific sequences that interact with nucleoli (Van Koningsbruggen et al., 2010) disturbance of the chromosome territories in this area could have wide ranging effects.

Moreover, it is possible that the relatively large chromosomal regions involved in Robertsonian translocations, or the involvement of chromosome fragments adjacent to the centromere, may be particularly disruptive to the positioning of chromosomes on the mitotic spindle, increasing the risk of chromosome malsegregation, especially in the absence of checkpoints and other corrective mechanisms.

The risk of any given chromosome being abnormal at the cleavage stage was 0.065 for the Robertsonian translocation carriers. This corresponds to an increase in the relative risk of aneuploidy of 1.41 per chromosome. However, since many embryos are abnormal for several chromosomes, the probability of abnormality at the level of the embryo (rather than at the level of the chromosome) is not increased as dramatically. Of 1,861 embryos in eligible control groups 1,185 (63.68%) were abnormal for one or more chromosomes (excluding embryos in which aneuploidy only affected chromosomes involved in the Robertsonian translocation in the corresponding patient). This compares to a 69.81% aneuploidy rate in the embryos of Robertsonian translocation carriers (again excluding those with abnormality affecting the translocated chromosomes only). Thus, the relative risk of a Robertsonian translocation carrier producing an abnormal cleavage stage embryo, due to an error unrelated to their constitutional rearrangement, is 1.096. In other words, out of every 11 euploid zygotes produced by a Robertsonian translocation carrier one is expected to become abnormal during the cleavage stage due to the ICE.

As expected, chromosome abnormality rates increased significantly with advancing female age in samples from patients with chromosome rearrangements and also in those derived

from controls. Increasing age did not seem to influence the apparent ICE seen in samples from Robertsonian translocation carriers, nor the lack of ICE observed in the carriers of inversions and reciprocal translocations.

In most cases, the additional aneuploidies caused by the ICE were of types incompatible with development beyond the first few days of life, producing embryos that would fail to implant or result in an early miscarriage. Indeed, the fact that a significant increase in aneuploidy rate was only seen at the cleavage stage and not in blastocyst stage embryos suggests that many of the abnormal embryos, or at least the aneuploid cells produced as a consequence of an ICE, are rapidly eliminated. Nonetheless, it is conceivable that some abnormalities resulting from the ICE could produce late miscarriages or the birth of children with severe congenital malformations.

Although the incidence of a chromosomally abnormal birth is only likely to be slightly elevated, the increase in the mortality of early embryos may have more significant consequences, impacting fertility, particularly for patients using assisted reproductive techniques in combination with PGD. For Robertsonian translocation carriers undergoing IVF, preimplantation genetic diagnosis using a comprehensive chromosome screening technique, capable of detecting aneuploidy affecting any chromosome is highly advisable and this is in agreement with other studies (Fiorentino et al., 2011; Treff et al., 2011). Older PGD methods utilizing FISH, which focus on analysis of the rearranged chromosomes only, should be discouraged, since they will fail to detect abnormalities affecting other chromosomes, arising as a consequence of an ICE.

In conclusion, an ICE is likely to affect carriers of Robertsonian translocations and may contribute to higher rates of abnormal embryos, resulting in a small increase in the risk of miscarriage and birth of children with congenital abnormalities and a potential reduction in fertility. These possibilities should be considered when counselling patients about the risk of abnormal pregnancy following natural conception or the likelihood of producing embryos suitable for uterine transfer during cycles of PGD. Further research to determine the mechanism by which rear-

ranged chromosomes disrupt the process of mitotic chromosome segregation should be encouraged.

7.4 Clinical outcomes for patients undergoing IVF combined with comprehensive chromosome screening

The hypothesis that chromosome screening could lead to improved IVF pregnancy rates by assisting in the identification and transfer of viable embryos (i.e. PGS) seems logical. As shown in the current investigation and in multiple preceding studies, aneuploidy is extremely common in human oocytes and preimplantation embryos. Additionally, numerous analyses of tissue derived from miscarriages have confirmed the lethality of chromosome abnormalities. However, despite these facts, several randomised trials employing PGS failed to show any improvements in IVF outcome following cleavage stage embryo biopsy and FISH analysis (Staessen et al., 2004, Mastenbroek et al., 2007). It has been suggested that the disappointing results from earlier randomised trials might have been a consequence of technical deficiencies and biological issues. For example, poor embryo biopsy technique may lead to reduced embryo viability, while the limitations of FISH result in imperfect detection of aneuploid embryos. In terms of biological problems, cleavage stage chromosomal mosaicism is probably of most relevance, potentially reducing diagnostic accuracy. For these reasons, the current work focused on methods allowing analysis of all 24 chromosomes and assessed blastocysts as well as cleavage stage embryos.

Comprehensive chromosome screening of blastomeres (biopsied from cleavage stage embryos) and TE cells (biopsied from blastocysts) was performed for couples with one or more of the following indications: advanced maternal age (AMA); a history of recurrent miscarriages (RM); repeated IVF failure (RIF). The decision to examine embryos either at the cleavage stage, or a couple of days later at the blastocyst stage was dependent on the referring IVF clinic and also on the quality of the embryos. The advantage of biopsying and cytogenetically analysing blastomeres is that it enables a larger number of embryos to be assessed, compared to the blastocyst stage. However, various studies have shown that this stage of development is characterised by elevated rates of aneuploidy, as well as mosaicism, potentially compromising diagnostic accuracy (Wells

and Delhanty, 2000; Voullaire et al., 2000; Mantzouratou et al., 2007; Vanneste et al., 2009; Gutierrez-Mateo et al., 2011). Furthermore, in recent years concerns have been raised over the impact of embryo biopsy at the cleavage stage (Van de Velde et al., 2000; Cohen et al., 2007; Goossens et al., 2008). Results from previously published studies indicate that rates of aneuploidy and mosaicism are lower amongst blastocysts than at other preimplantation stages (Fragouli et al., 2008; 2011). Moreover, embryos that are capable of successfully negotiating the first cellular differentiation into TE and ICM are considered to be of the highest implantation potential.

Both metaphase and aCGH performed equally well yielding results for 88.3% (701/794) of blastomeres and 93.5% (1026/1097) for TE samples. These results are comparable with efficiencies recently reported by other groups using aCGH (Gutierez-Mateo et al., 2011; Fiorentino et al., 2011; Yang et al., 2012). In agreement with other investigations (Sandalinas et al., 2001; Fragouli et al., 2008; Santos et al., 2010) a significant decrease in aneuploidy rates was observed when cleavage stage embryos were compared to blastocysts (Fisher's exact test, $P < 0.0001$, $P = 0.0002$ and $P < 0.0001$ for AMA, RM and RIF, respectively). Blastocyst aneuploidy rates for the three patients groups were 18-27% lower than those seen at the cleavage stage. Moreover, 36.3% of cleavage stage embryos had 3 or more chromosome abnormalities (complex) whereas only 9.1% of blastocyst stage embryos had complex abnormalities (Fisher's exact test, $P < 0.0001$).

The adverse effect of advancing female age on oocyte and preimplantation embryo aneuploidy, reported previously by many studies (e.g Angell et al., 1983; Delhanty et al., 1993; Munné et al., 1993; Iwarsson et al., 1999; Wilton et al., 2003; Fragouli et al., 2008; 2010; 2011), was also obvious in the current results. In order to examine potential differences between embryos from patients referred for PGS for different reasons, this confounding factor had to be taken into account. For this reason, data from the three different indications were sub-divided according to female age into a younger group which included women between 27 and 36 years and an older group which included women who were 37 years or more. Taking into account the dif-

ferences in aneuploidy rates observed at the cleavage and blastocyst stages necessitated a further subdivision of the data according to the developmental stage assessed.

Fragouli et al (2010) examined blastocysts from patients with RIF via metaphase CGH (average female age 38.4 years) and reported an aneuploidy rate of 45.2%. Similarly, Voullaire and colleagues (2007) performed metaphase CGH for the investigation of cleavage stage embryos also generated from patients with history RIF (average female age not mentioned) and reported a somewhat higher aneuploidy rate of 55%. The authors reported that aneuploidy rates for embryos with 1-2 errors were similar for RIF patients and those with no history of RIF. However, the proportion of embryos with multiple chromosome abnormalities was significantly higher for RIF patients (irrespective of female age). This suggests that the reduced embryo implantation rates seen in patients who have experienced several unsuccessful IVF cycles might, in some cases, be related to a tendency to generate an elevated quantity of grossly abnormal cleavage stage embryos.

The data obtained during this part of the project is in line with that of both Fragouli et al., (2010) and Voullaire et al., (2007). Specifically, the aneuploidy rate scored in cleavage stage embryos from the RIF group was 81.5% (average female age 36.8 years), while that scored at the blastocyst stage was 54% for an (average female age of 37.5 years). The proportion of abnormal cleavage stage embryos with 1 or 2 errors from RIF patients was considered in comparison with those from AMA patients (average female age 40.8 years). The AMA patients have no history of unsuccessful IVF treatment or of miscarriage. Their only indication for chromosome testing is female age. The frequency of those embryos was similar among the AMA and RIF patient groups (43.2% and 42.3%, respectively). However, the data from the current study was not in agreement with Voullaire et al's (2007) data regarding embryos with complex abnormalities, as the frequency of embryos carrying complex errors was higher in the AMA, compared to the RIF group (45% vs. 39%) whereas their data suggested an increase in the frequency of embryos with such errors

in the RIF group compared to patients with other indications. This could be due to the small number of cases included in their study with indications other than RIF (22 patients with RIF and 6 patients with other indications).

Following on from their work using metaphase CGH, Voullaire et al (2007) suggested that the origin of complex aneuploidy is generally mitotic. Mantzouratou et al (2007) had also examined embryos from RIF, AMA and RM patients, assessing 438 embryos on day-5 or -6, using FISH (chromosomes (13, 15, 16, 18, 21 and 22). They concluded that the overall aneuploidy rate was similar between the different groups of patient, but most of the errors detected in RIF patients was of post-zygotic rather than meiotic origin. Moreover, Kay et al. (2006) found that polymorphism of the tumor suppressor gene p53 is associated with repeated implantation failure with an unknown mechanism. Therefore, the mechanism leading to impaired implantation in this group of patients is not known and differences observed in our study with those published studies (Voullaire et al., 2007) could be explained by the differences in patient groups examined in the two studies, although they are both suffering from repeated implantation failure.

In order to investigate the aneuploidy rate in the RIF group and whether increases in specific types of aneuploidy (e.g. complex errors) are found in this group, embryos from 'older' RIF patients were compared to embryos from AMA patients of 37 years or more. No differences were observed between these two groups in terms of aneuploidy at the cleavage or blastocyst stages (average female age was identical for the groups compared). At the cleavage and blastocyst stages, both groups had similar frequencies of embryos with complex errors.

It has been estimated that a quarter of women trying to achieve a pregnancy have at least 1 spontaneous abortion and that half of pregnancies miscarry before birth (Hassold et al., 1978; Warren and Silver, 2008). Most miscarriages are sporadic and are associated with a relatively low recurrence risk (greatly influenced by maternal age). However, 0.5 to 1% of couples trying to conceive suffer from recurrent miscarriages, being defined as the early loss of 3 or more

pregnancies (Warren and Silver, 2008). Recurrent miscarriages have been attributed to various aetiologies ranging from immunological, endocrine, environmental, uterine anatomical abnormalities, and genetic abnormalities. Cytogenetic abnormalities are thought to account for 50-70% of sporadic spontaneous abortions and around a third of pregnancy losses in women with recurrent miscarriages. Cytogenetic analysis of products of conception has shown that trisomies account for 60% chromosomally abnormal miscarriages, monosomies for 20% and polyploidies for another 20% (Silver and Branch; 2007). Hassold et al. (1980) studied two successive abortions from 40 couples (29.9 ± 5.7 years) and found that both abortions were karyotypically normal in 21/40 women, while in 9 women both miscarriages were abnormal.

During this project, the aneuploidy rate scored for day-3 embryos generated from RM patients (average female age 38 years) was 71.7%. Embryos with 1-2 errors accounted for 40.7% of all embryos and those with complex errors accounted for 31%.

Moreover, aneuploidy rate observed in cleavage stage embryos from younger RM patients (average female age 34.2 years) was 61% which was lower than that observed from younger RIF patients (average female age 32.8 years) at 73.9%, but no statistical significance was observed (Fisher's exact test, $P = 0.2$). The rate of aneuploid embryos with 1-2 errors from younger RM patients was 28%, which is somewhat lower than that observed in cleavage stage embryos from younger RIF (45%), this difference was, however, not statistically significant.

Euploidy rate in cleavage stage embryos generated from older RM patients (average female age 40.3 years) were significantly higher than those from AMA group (21% vs. 12%, respectively, $P = 0.035$), but no different from those generated from older RIF patients (average female age 40 years) (14.3%). Aneuploid embryos with 1-2 errors from older RM patients accounted for 48% of all observed embryos, which was higher (although not significantly) than those from AMA or older RIF groups with 1-2 errors (43.4% and 38.1%, respectively).

Surprisingly, the proportion of cleavage stage embryos with complex abnormalities from older RM patients was similar to that seen in young RM patients (30.2% vs. 32.2%, respectively) and significantly lower than the aneuploidy rates observed from older RIF and AMA embryos (47% and 45%, respectively; *P* = 0.01 and 0.02). Patients with recurrent miscarriages can usually conceive naturally and their reasons for seeking PGS are different to those of patients with RIF or AMA.

A recent study by Najafi et al. (2012) compared levels of endothelial nitric oxide synthase (eNOS) which plays a role in embryo implantation between women with recurrent miscarriages or unexplained infertility with a control group and found increased expression of eNOS in the recurrent miscarriage and unexplained infertility groups compared to the control group and suggested an adverse effect of the increased levels of nitric oxide on the receptivity of the endometrium and embryo implantation. Moreover, Fragouli et al. (2011) compared aneuploidy rate observed in first and second polar bodies from patients with AMA, RM and RIF and Mantzouratou et al. (2007) examined cleavage stage embryos generated from patients with AMA, RM and RIF and both studies reported the least aneuploidy rate in the RM group. There is no question that the RM patients represent a heterogeneous group and the causes of miscarriages are not always due to chromosomal abnormalities. This might explain the differences seen when these patients are compared to those with different indications for PGS; increased euploidy levels and decreased rate of complex errors in the older group at the cleavage stage.

Aneuploidies detected in cleavage and blastocyst stage embryos from patients with AMA, RM and RIF were found to affect all chromosomes. Data obtained from analysis of cleavage (Delhanty et al., 1996; Wells and Delhanty, 2000; Mantzouratou et al., 2007; Gutierez-Mateo et al., 2011) and blastocyst stage (Fragouli et al., 2010; Yang et al., 2012) reported that chromosomes equal to chromosome 13 or smaller are more frequently affected by aneuploidy. The current data confirms these previous findings, but with some exceptions for the different PGS indications. Spe-

cifically, larger chromosomes were seen to be frequently affected by aneuploidy events in embryos produced from younger RIF patients (chromosomes 12, 4 and 1 each had a 7% aneuploidy frequency at the blastocyst stage, Figure 6-10) and younger RM patients (chromosome 12 at the cleavage stage with 6% frequency and chromosome 4 with 8.3% at the blastocyst stage, Figures 6-6 and 6-10). In the embryos generated by the older RIF patients, chromosome 1 was scored frequently aneuploid at the cleavage stage (5.8%), whereas malsegregation errors affecting chromosome 2 were seen in 6.9% of the blastocysts from older RM patients. In the majority of cases, aneuploidy of such large chromosomes is expected to be incompatible with formation of a clinical pregnancy.

Chromosome losses were reported to be common in polar bodies (Vialard et al., 2007; Fragouli et al., 2010; Handyside et al., 2011), outnumbering chromosome gains, and leading to a predicted excess of trisomies at the cleavage and blastocyst stage. However, previous studies at the cleavage (Daphhnis et al., 2008; Gutierez-Mateo et al., 2011) and blastocyst stages (Coonen et al., 2004; Yang et al., 2012) reported similar rates of losses and gains, or even a small excess of losses (i.e. monosomy). In the current study, similar observations were made at the cleavage stage for all three investigated patient groups, regardless of female age. This discrepancy between the expected excess of trisomies predicted from PB analysis and the observed similar rate of losses and gains seen in embryo analysis is most likely attributed to mechanisms leading to post-zygotic errors in embryos which are mainly anaphase lagging leading to chromosome losses (Coonen et al., 2004; Daphnis et al., 2008). However, at the blastocyst stage, embryos generated by RIF patients (younger and older) had a skewed excess of trisomies compared to monosomies (monosomy:trisomy ratio was 0.6:1 for younger group and 0.84:1 for the older group). Previous studies have highlighted that patients with RIF have altered frequencies of certain polymorphisms in the TP53 gene (Kay et al. 2006) and also an increased incidence of post-zygotic errors (Mantzouratou et al., 2007; Voullaire et al., 2007). It is possible that embryos with monosomies are more sensitive to such defects, resulting in their demise during preimplantation development, or

perhaps leading to an increased incidence of chromosome gains during first mitotic divisions of embryonic development. All these are highly speculative suggestions and further studies in the molecular mechanisms that lead to chromosome errors in RIF patients are essential to determine the true basis of such events.

The frequency of aneuploidies capable of implanting and forming a clinical pregnancy (such as trisomies 13, 16, 18, 21, 22, and sex chromosome imbalances) was 50% higher amongst the embryos of RM patients compared with those referred for RIF. This overrepresentation in the embryos of RM patients might explain the increased incidence of early abortions in the RM group (i.e. abnormalities in RM embryos are often compatible with implantation whereas those in RIF embryos are typically of greater lethality). Comparing cytogenetic results from cleavage stage embryos with those obtained from blastocysts it is clear that culturing embryos to the blastocyst stage reduces the risk of aneuploidy, but does not come close to eliminating it. This is especially true for the types of aneuploidy compatible with pregnancy formation, which have little if any negative impact on preimplantation development and no significant effect on blastocyst morphology (as discussed earlier).

Interestingly, partial (segmental) chromosome abnormalities, presumably resulting from chromosome breakage followed by deletions or duplications were seen both at the cleavage and blastocyst stages. They mostly affected the larger chromosomes (1-12), although segmental anomalies affecting smaller chromosomes (16, 19, 20 and X) were also seen. Such partial abnormalities have also been reported in other investigations employing CGH or aCGH to examine the embryonic chromosome complement (Wells and Delhanty, 2000; Voullaire et al., 2000; Voullaire et al., 2007; Vanneste et al, 2009; Fragouli et al., 2010; Gutierez et al., 2011; Yang et al., 2012). Interestingly, in the dataset presented here, chromosome breakages (proportion of abnormalities of segmental type) were more common in embryos of younger patients with RM (10% on day-3 and 13% on day-5) and RIF (10% on day-3 and 11% on day-5 embryos) compared to older patients

where they were observed in ~6% of cleavage stage embryos from AMA (day-3 and day-5), RM (7% day-3 and 12% day-5 embryos) and RIF (6% day-3 and 8% day-5 embryos). Vanneste et al. (2009) reported a high frequency of segmental errors in cleavage stage embryos generated from young women (<35 years old) which is in agreement with our findings. The observed decline in the proportion of segmental errors with age is probably due to the increased incidence of whole chromosome errors with advancing female age with constant incidence of segmental errors and the overall observation is a decline in their incidence.

The clinical outcomes following comprehensive chromosome analysis were relatively encouraging for patients of all indications (AMA, RM and RIF), especially after blastocyst biopsy. Hence, the Implantation rate (IR) was 25% after day-3 analysis followed by day-5 transfer, 61% after day-5 analysis followed by day-6 transfer and 57% after day-5 and/or -6 analysis followed by cryopreservation and transfer in an unstimulated cycle. The study reported in this thesis is purely observational and makes no claims concerning the efficacy of PGS using comprehensive chromosomal methodologies. No appropriate control group for assessment of the impact of PGS on clinical outcomes was available. In order to accurately evaluate the clinical utility of PGS using methods such as aCGH a well-designed randomised trial would be required. In the last year such a trial has been published by Yang and colleagues (2012). As discussed above, they reported confirmed that PGS, performed at the blastocyst stage for good prognosis IVF patients, resulted in a significant increase in ongoing pregnancy rate per cycle (Yang et al., 2012). The pregnancy rate observed by Yang et al (70.9%) was higher than that of the current study, which is expected given the superior prognosis of their cohort of patients.

The pregnancy rate per cycle for AMA couples following day-3 analysis was approximately 9%, little different from published pregnancy rates for patients in this age group, but increased to 41% following day-5 analysis, a surprisingly good Figure given the very poor prognosis of this patient group. The overall pregnancy rate (per cycle) achieved for RM patients was 37.5% with an

implantation rate of 57.1% following cleavage stage screening (average maternal age 36 years) and 76% and 69%, respectively following blastocyst screening (average maternal age 36.3 years). While no clear conclusion can be drawn from this uncontrolled data, it is encouraging to note that the implantation and pregnancy rates are considerably higher than typically observed for such patients (ESHRE PGD consortium, 2012).

The expected rate of miscarriage for the RM group was calculated using Brigham's formula, which takes into account the female age and the number of prior miscarriages for each patient. The expected miscarriage rate for future pregnancies was determined to be 31% for the RM patients who had PGS performed at the cleavage stage and 27% for those who had their embryos screened at the blastocyst stage. However, miscarriages were only observed in 16% of cases following day 3 analysis and 18% following day 5 analysis, which represented a significant decline following day 3 analysis with the decline not significant following day 5 analysis (Chi square test, P = 0.03 and 0.17, respectively). Therefore, comprehensive chromosome screening provides for some of this heterogeneous group of infertile patients a reduced miscarriage rate and an increased chance to have a healthy baby.

When examining the effect of age on pregnancy and implantation rates after day-3 analysis, it was observed that younger patients with RM achieved a 60% pregnancy rate per cycle and a 37.5% implantation rate (average maternal age 33.6 years). Surprisingly, for older RM patients, the pregnancy rate per cycle was 27.3% and implantation rate was 83.3% (average maternal age 39.7 years). However, it has to be noted that 40% of cases less than 37 years did not have any normal embryos to be transferred compared to almost three quarters of cases of 37 or more years. The sample size is too small to draw any firm conclusions as only 5 patients were included in the younger and 11 in the older patient groups..

Following day-5 analysis pregnancy rate per cycle for RM patients was 100% (as all tested patients had at least one normal embryo and achieved a pregnancy) and implantation rate was

67% for patients less than 37 years (average maternal age 32.3 years). This compares to pregnancy rate per cycle and implantation rate of 59% and 72%, respectively for patients over 37 years (average maternal age 39.7 years). The miscarriage rate was higher in the younger group following day 3 and day 5 analysis (33% and 25% respectively) compared to that in the older group (0% and 10% respectively). A possible explanation for this is that the cause of recurrent miscarriages in the younger patients is not due to chromosomal abnormalities, but due to other factors such as the excessive presence of eNOS in the endothelium which has been linked with recurrent miscarriages (Najafi et al., 2012) or the presence of genetic mutations or polymorphisms such as that reported in the methylenetetrahydrofolate reductase (MTHFR) gene which was linked with patients with RM (Mtiraoui et al., 2006; Nair et al., 2012).

The most recent European Society of Human Reproduction and Embryology data collection (between December 2008 and October 2009) (Goossens et al., 2012) reported a 24.3% pregnancy per cycle and 30.1% per transfer for RIF patients with an average maternal age of 35.1 years and all the PGS cases were performed on cleavage stage embryos with 21.7% implantation rate. Moreover, a 15% implantation rate and 21% pregnancy rate was reported by Wilton et al. (2003) where cleavage stage embryos generated by patients with RIF were screened using CGH (average maternal age 34 years). In this part of the project it was found that the pregnancy rate per cycle in younger patients with RIF (average maternal age 31.7 years) was 40% following cleavage stage comprehensive chromosome screening with an implantation rate of 33.3%. These results represent an encouraging finding for these patients, indicating that PGS using comprehensive chromosome screening methods might be beneficial for them. Moreover, the pregnancy was higher following day 5 embryo screening for the younger and older RIF groups (67% and 58%, respectively) and so was the implantation rate (50% and 56%, respectively). These results show the usefulness of screening embryos at the blastocyst stage in comparison to the cleavage stage, as for all patient groups the pregnancy and implantation rates were higher following blastocyst analysis compared to cleavage stage analysis. This is likely due to the lower mosaicism rate

amongst blastocysts (as shown previously), less detrimental effects on the embryo of biopsy and loss (via embryonic arrest) of embryos incapable of forming blastocysts (Cohen et al., 2007; Goossens et al., 2008; McArthur et al., 2008).

The higher implantation and pregnancy rates achieved following blastocyst compared to cleavage stage analysis may be due to negative effects of blastomere biopsy leading to reduction in embryo viability (Cohen et al., 2007). On the other hand, the biopsy of an average of 5 trophectoderm cells from embryos at the blastocyst stage has been reported to be less detrimental and the survival rate has been reported at almost 100% of biopsied embryos (McArthur et al., 2008; Schoolcraft et al., 2009).

Chromosome screening on day-5 may offer the most appropriate stage to choose the embryo with highest implantation potential, with fewer errors due to mosaicism, less risk of biopsy damage, and embryos that have already successfully achieved several developmental milestones. However, this approach is complicated by the requirement to either cryopreserve the embryos following biopsy or to perform an extremely rapid test allowing a fresh transfer to be performed on day-6 of the same cycle. Although embryo cryopreservation is not without some risks, these have been lessened by the introduction of vitrification methods (Cao et al., 2009; Zhang et al., 2011). Furthermore, there is some evidence that uterine receptivity may be superior in unstimulated cycles compared to those in which ovarian stimulation has just occurred and therefore embryo cryopreservation, with transfer in a subsequent cycle, may lead to enhanced implantation rates (Shih et al., 2008). A study by Schoolcraft et al (2010) during which embryos were biopsied and vitrified on day-5, with the biopsied cells analysed by metaphase CGH and embryos transferred in a later unstimulated cycle, reported very high implantation and pregnancy rates which were attributed to a combination of enhanced embryo selection and optimised uterine receptivity. While a clinical strategy involving blastocyst biopsy and vitrification appears to be valid, fresh day-6 transfer following day-5 biopsy is also increasingly widely used. Previous studies

suggested that transfer of embryos which formed expanded blastocysts on day-5 and were transferred on day-6 is associated with similar pregnancy rates to more traditional transfers performed on day-5 (Elgindy and Elsedeek, 2012). In the current work, the pregnancy rate per transfer was compared between fresh day-6 transfer (in the same stimulated cycle) and vitrified/warmed embryos transferred in a subsequent cycle and no significant differences were found (77% vs. 83%, respectively). Similarly, implantation rates were also the same for fresh and vitrified cycles (61% vs. 57%, respectively). Surprisingly, when pregnancy rate was considered per cycle, rather than per transfer, it was found to be significantly lower in the group where fresh transfer took place (27% compared to 65% for vitrified/warmed cycles; $P = 0.0003$). This difference might be attributed to the number of embryos available for biopsy in the two groups. Hence, for the fresh transfer group only embryos that formed blastocysts on day-5 were biopsied and subsequently examined. The average number of blastocysts in this group was 3.1. More embryos were generated and analysed in the vitrified/warmed group (average number of embryos 5.4), as biopsy took place on both day-5 and day-6.

7.5 Future perspectives on aneuploidy detection and embryo selction

The development of more advanced non-invasive techniques able to assist in selecting the embryo(s) with the highest implantation potential is rapidly evolving. One such non-invasive approach involves the transciptomic analysis of follicular cells, granulosa and/or cumulus, to obtain information on the quality of the oocyte to be fertilised and the implantation potential of the resulting embryo. The gene expression analysis, especially of cumulus cells, has provided very interesting data concerning the follicular environment of e.g. mature and immature, and normal and aneuploid oocytes (Ouandaogo et al., 2011; Fragouli et al., 2012). The transcriptomic analysis of cumulus cells is also being applied clinically to select competent oocytes, able to create embryos with high implantation potential (Assou et al., 2008). Other non-invasive approaches include the analysis of embryo metabolism, as well as the identification of proteins and other molecules secreted by the embryo in the surrounding culture medium (Botros et al., 2008 ; Katz-Jaffe et al., 2010). Metabolomic profiling of embryos has also been applied clinically, but with mixed results, failing to yield any benefits in a recent randomised controlled trial (Hardarson et al., 2012).

Recently, the use of non-invasive time-lapse imaging of human embryos in combination with comprehensive chromosome screening in order to find a pattern between blastomere behaviour and ploidy during the first two cleavage divisions showed that all euploid embryos followed a precise set of timings (time of division, length of division, etc) to the four-cell stage compared to only 30% of the aneuploid embryos (Chavez et al., 2012). Moreover, Campbell et al. (2013) used a time-lapse imaging system (Embryoscope; FertiliTech) to aseess specific variables such as time, in hours post insemination (hpi), for start of comapction (tSC), start of blastolation (tSB) and full blastocyst formation (tB) and reported a significant delay of embryos with complex errors compared to euploid embryos in (tSC), (tSB) and (tB). These results indicate the possibility of identifying chromosomally abnormal embryos with complex errors but not those with single or double errors, especially those capable of implanting. Moreover, the application of early embryo

viability assessment (EEVA; Auxogyn) as blastocyst formation predictor provides another non-invasive imaging approach (Conaghan et al., 2013) and more studies on the effectiveness of this technique in enhancing implantation rate and reducing miscarriage rate is required. Therefore, further assessment of time-lapse imaging is essential and could ultimately provide a non-invasive method to choose normal embryos with high implantation potential.

On the other hand, studies on cytoplasmic movements in freshly fertilized mouse oocytes showed a correlation between the frequency and velocity of these movements and the embryo potential to develop to birth (Ajduk et al., 2011). Thus, the introduction of this aspect in human oocytes and embryos might provide a very useful and cost-effective tool that can predict embryo viability.

7.6 Conclusion

To conclude, results from this project have shown that CGH and aCGH can accurately determine the molecular karyotype of human blastocysts through a biopsy of an average of 5 cells from the trophectoderm layer. Moreover, it has been confirmed that culturing embryos to the blastocyst stage does not eliminate the risk of aneuploidy that, even at this late stage of preimplantation development, can still potentially affect any chromosome. The data presented is the most detailed description of the chromosomal status of the human blastocyst stage to date. Previous analyses of the final embryonic stage prior to implantation had only been able to assess small numbers of embryos (using chromosome banding methods) or had only been capable of testing a limited number of chromosomes (using FISH). The results described in this thesis indicate that some selection against aneuploid embryos occurs during the preimplantation period, likely beginning after activation of the genome at the 4-8 cell stage, but also clearly demonstrates that majority of aneuploidies of both meiotic and mitotic origin are capable of persisting until the blastocyst stage. Since most of the chromosome abnormalities detected are of varieties never observed in products of conception or prenatal samples, it seems likely that most affected embryos fail at the time of implantation or shortly thereafter.

Meiotic abnormalities will affect all cells of the embryo while mitotic abnormalities affect a subset of the cells and lead to mosaicism, a phenomenon of great relevance to PGD and PGS accuracy rates. Mosaicism was detected at the blastocyst stage, but was generally less common than at the cleavage stage. Of the mosaic blastocysts seen in this study, most were aneuploid in every cell and of those that were a mixture of diploid and aneuploid cells only a small minority (6%) had more than half of their cells normal. This provides some reassurance that a TE biopsy will be predictive of embryo cytogenetic status in the vast majority of occasions. However, the data also indicates that the accuracy of embryo testing using metaphase CGH and aCGH is not

100% and some errors are to be expected. This is an important point to note during patient counselling.

The work presented demonstrates that cleavage stage embryo morphology (the primary factor currently used to determine which embryo should be transferred to the uterus) is not a reliable indicator of chromosomal status. The incidence of aneuploidy was high amongst all morphological grades considered appropriate for transfer (grades 1, 2 or 3). Little difference in aneuploidy rate was seen for embryos of the highest and lowest morphological grades. An over-representation of aneuploid embryos was only observed in embryos of very poor quality which are rarely chosen for transfer (grade 4). On the other hand, a weak correlation between blastocyst morphology and aneuploidy was identified, the proportion of normal embryos declining with decreasing embryo quality. However, abnormal embryos potentially capable of implanting and forming a viable pregnancy (trisomy 13, 16, 18, 21, 22 and sex chromosome aneuploidies) had morphologies indistinguishable from normal embryos. Therefore, transferring better quality blastocysts does not eliminate the risk of aneuploidy, especially of types capable of forming clinical pregnancies. The only reliable way to avoid the transfer of chromosomally abnormal embryos remains to undertake biopsy, ideally performed at the blastocyst stage, followed by comprehensive chromosome screening.

Interestingly, until the cleavage stage, female embryos were seen to grow faster than male embryos. However, after embryonic genome activation, male embryos caught up with, and ultimately overtook females. The disparity in embryo growth is unlikely to reflect any difference in competence between male and female embryos but indicates that male embryos reach the final stages of in vitro development earlier than female embryos. The underlying reason for the difference in developmental rate is unknown, but may be related to X-chromosome dosage compensation in females or Y-chromosome growth factor genes in males. Since IVF clinics tend to preferentially select the most developmentally advanced embryos for transfer to the uterus,

there is a possibility of cycles involving culture to the blastocyst stage causing a sex ratio skewed in favour of males. A deviation from a 1:1 sex ratio has been confirmed amongst children conceived following blastocyst transfer. Infertile patients having IVF and extended embryo culture should be counselled that there is an increased likelihood of having male offspring.

Aneuploidies for chromosomes in groups D-G (smaller size chromosomes) and X/Y chromosomes were more affected by female age than other chromosomes. This suggests that mechanisms leading to chromosome malsegregation are different in younger and older patient groups. It is thought that chiasmata (sites of meiotic recombination on chromosomes), which tend to be present in fewer numbers on smaller chromosomes, are influenced by age. Also, the number of cohesin molecules binding together the constituent chromatids may be related to chromosomal length. Age-related decline in cohesin may therefore be particularly relevant to small chromosomes. These factors may explain why smaller chromosomes appear to be more prone to aneuploidy with advancing female age.

The pregnancy rates per cycle following PGS for patients with AMA, RM and RIF following cleavage stage screening was considerably lower than following blastocyst stage screening. Pregnancy rates are generally less for IVF cycles that involve cleavage stage transfer compared with those in which transfer is carried out at the blastocyst stage. However, this was exacerbated after PGS by large numbers of cycles in which no normal embryos were detected. Despite poorer results for cleavage stage PGS, pregnancy rates were still as good as or higher than those generally expected for such a patient group. Although no randomised trial was attempted during the course of this investigation, results from blastocyst PGS were particularly encouraging, associated with high pregnancy rates and a low of incidence miscarriage. Outcomes for IVF cycles involving blastocysts biopsy and aCGH appeared to be superior to those typically expected for similar patients in the absence of chromosome screening. These promising, but preliminary results now

need to be verified in a well-designed randomised trial. However, one trial published in the last few months is supportive of the findings reported here (Yang et al., 2012).

Even if the efficacy of PGS at the blastocyst stage, using comprehensive chromosome analysis methods such as aCGH, is confirmed there still remain questions about the most appropriate biopsy strategy to employ. According to the available data from this project, no differences were observed in pregnancy and implantation rate per transfer following day-5 biopsy with fresh day-6 transfer compared to day-5 biopsy with vitrified/warmed transfer in a subsequent cycle. However, the pregnancy rate per cycle was lower for cases were fresh transfer was performed as a result of lower numbers of embryos available for biopsy early on day-5 (a necessity for fresh day-6 transfer). In the case of cycles involving vitrified/warmed embryos, biopsy can be carried out throughout day-5 and even on day-6. This maximises the number of embryos available for analysis, although it comes with a small risk to each embryo associated with the cryopreservation procedure.

The current work demonstrates that, as well as their use in PGS, CGH and aCGH offer an accurate method for detection of chromosome imbalances in embryos produced by carriers of a chromosome rearrangement. Indeed, the world's first births following the application of comprehensive chromosome analysis to embryos from translocation carriers occurred as a result of work presented in this thesis. Using a comprehensive chromosome screening method offers a universal method to detect abnormalities associated with rearrangements. As well as eliminating the time-consuming, expensive, patient-specific work-up that used to be necessary for diagnosis of such abnormalities using FISH, this approach also permits detection of embryos with unrelated, spontaneously arising aneuploidies, potentially reducing miscarriage rates and improving IVF success rates.

An unexpected and interesting finding from this work was that embryos produced by Robertsonian translocation carriers have a generalized increase in aneuploidy risk, known as in-

ter-chromosomal effect (ICE). In the main, this increase in aneuploidy appears to affect mitotic chromosome segregation, as female gametes from carriers of such rearrangements were shown to have a similar frequency of aneuploidy compared to the control group. As a result, of these findings carriers of chromosome rearrangements, especially Robertsonian translocations are advised to be counselled regarding their risks of aneuploidy unrelated to their rearrangement and, if choosing to have PGD, to undergo comprehensive chromosome screening to avoid the transfer of abnormal embryos.

In summary, this project has shown that the level of mosaicism declines gradually as the embryo develops to the blastocyst stage as well as the rate of aneuploidy. Blastocyst morphology provides a weak link to chromosome status of the embryo but cannot be relied on to select the embryo with highest implantation potential. Carriers of chromosome rearrangements, especially Robertsonian translocations, are at a higher risk of generating embryos with chromosome errors compared to the general infertile population. Mechanisms that lead to chromosome abnormalities in infertile couples of advanced maternal age, recurrent miscarriages and repeated implantation failure may differ and therefore lead to different patterns of errors in their embryos.

8. References

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9. Appendices

Appendix A

1% Agarose gel

1 g agarose gel (Invitrogen, UK)

1x Tris-Borate_EDTA (TBE) (Sigma Aldrich, USA)

70% formamide/2x SSC

10 ml double-distilled water

5 ml 20x SSC (pH = 7) (Fisher scientific, UK)

35 ml deionized formamide (Sigma Aldrich, USA)

50% formamide/2x SSC

2.4 ml double-distilled water

600 µl 20x SSC (Fisher scientific, UK) (pH = 7)

3 ml deionized formamide (Sigma Aldrich, USA)

2x SSC/0.1% NP-40

45 ml double-distilled water

5 ml 20x SSC (Fisher scientific, UK)

50 µl NP-40 (Sigma Aldrich, USA)

0.4x SSC/0.3% NP40

49 ml double-distilled water

1 ml 20x SSC (Fisher scientific, UK)

150 µl NP-40 (Sigma Aldrich, USA)

0.7x SSC/0.3% NP-40

48.25 ml double-distilled water

1.75 ml 20x SSC (Fisher scientific, UK)

150 µl NP-40 (Sigma Aldrich, USA)

2x SSC/0.05% Tween-20

899.5 ml double-distilled water

100 ml 20x SSC (Fisher scientific, UK)

500 µl Tween-20 (Fisher scientific, UK)

1x SSC

475 ml double-distilled water

25 ml 20x SSC (Fisher scientific, UK)

0.1x SSC

995 ml double-distilled water

5 ml 20x SSC (Fisher scientific, UK)

Carnoy's fixative (3:1 Methanol:Acetic acid)

15 ml Methanol (Sigma Aldrich, USA)

5 ml Acetic acid (Sigma Aldrich, USA)

0.01 N HCl/ 100 µg/ml Pepsin

49 ml double-distilled water

0.5 ml 1 N HCl (Sigma Aldrich, USA)

0.5 ml 10 mg/ml Pepsin (Sigma Aldrich, USA)

1% Paraformaldehyde

49 ml double-distilled water

1.34 ml Paraformaldehyde (Sigma Aldrich, USA)

Appendix B

Table B-1: Patient details and cleavage stage embryo karyotypes

Patient	Age	Embryo no.	Embryo grade	Embryo cell number	aCGH result	Embryo characterisation
1	27	1	2	8	No result	Undiagnosed
		2	2	9	43,XY,-5,+7,-9,-10,-11,+12,-13,+14,-15,+17,+18,-20,-22	Abnormal
		3	2	8	46,XX	Normal
		4	2	9	46,XY,+16q	Abnormal
		5	2	8	45,X0	Abnormal
		6	2	6	49,XXY,+6,+13,+19p	Abnormal
		7	2	9	46,XX,+19p13.31_13.43	Abnormal
2	30	1	1	9	49,XX,-1,-2,+3,-11,+12,+16,+19,+20,+21	Abnormal
		2	1	9	44,XX,-8,-19	Abnormal
		3	1	11	47,XY,-16q,+19	Abnormal
		4	1	8	46,XY,+Xp	Abnormal
		5	1	8	41,X0,-1,-4,-18,-22	Abnormal
		6	2	7	49,XXYY,+1,+4,-13,+18,-20	Abnormal
		7	1	13	46,XY	Normal
		8	1	11	46,XX	Normal
		9	1	10	45,XY,-9	Abnormal
		10	1	8	46,XX,+13,-19	Abnormal
		11	1	8	45,XY,-16	Abnormal
		12	1	8	46,XX	Abnormal
		13	2	8	No result	Undiagnosed
3	31	1	2	6	46,XX	Normal
		2	2	9	50,YY,+3,+8,-12,+14,+15,+17,-18,-19,+20,+22,-X	Abnormal
		3	2	6	No result	Undiagnosed
		4	3	7	46,XX	Normal
4	33	1	2	8	No result	Undiagnosed
		2	3	8	No result	Undiagnosed
		3	3	8	No result	Undiagnosed
		4	3	8	No result	Undiagnosed
		5	2	8	46,XY	Normal
		6	3	8	45,XX,-8	Abnormal

4		7	2	8	No result	Undiagnosed
		8	2	8	No result	Undiagnosed
		9	3	8	No result	Undiagnosed
		10	3	6	43,XX,-3,-5,-6,+13,+16,-19,-20	Abnormal
5	34	1	4	8	46,XXX,+1,-5,+7,-8,-10,-14,+15,-17q,+19,-22	Abnormal
		2	4	8	43,XYY,-1,-2,+3,-4,+5,+6,+7,-8,-9,-10,-11,-12,-13,-14,+15,+16,+17,-18,-19,+20,-22	Abnormal
		3	3	8	47,XX,+13	Abnormal
		4	3	8	46,XX	Normal
		5	3	6	46,XX	Normal
		6	2	7	46,XX,+18p	Abnormal
		7	2	7	41,-2,-5,+7,-8,-10,+11,+12,-14,-15,+16,+20,-21,-X,-Y	Abnormal
		8	3	8	45,XY,-22	Abnormal
		9	3	8	45,XX,-22	Abnormal
		10	3	6	46,XY,+1q,+3q	Abnormal
		11	3	8	45,XY,+7p,-7q,-16	Abnormal
6	34	1	1	8	47,XX,+22	Abnormal
		2	1	6	46,XX	Normal
		3	1	8	45,XX,-21	Abnormal
		4	1	8	46,XY	Normal
		5	1	4	46,XX,+1p/q,-3,-5(x2),+6,+7,+8,+10,+12,-13,-14,-18,+19,-20	Abnormal
		6	1	8	46,XX	Normal
7	34	1	2	7	48,XY,-9p,+14,+20	Abnormal
		2	4	8	46,X0,-9,+16,+22	Abnormal
		3	4	9	43,X0,-20,-22	Abnormal
		4	3	8	48,XX,+7,+19	Abnormal
		5	4	8	45,XY,-8	Abnormal
		6	2	8	45,XX,-4	Abnormal
8	34	1	2	9	46,XX	Normal
		2	2	10	47,XX,+22	Abnormal
		3	2	9	No result	Undiagnosed
		4	2	7	45,XX,-22	Abnormal
8		5	2	7	46,XY	Normal
		6	2	8	47,XX,+21	Abnormal

		7	2	9	46,XXYY,+1,-2,-3,-4p,-6p,+7,-11,-12,+21,-22	Abnormal
		8	1	7	46,YY,+1,-2,+12q,-13,-15,-18,+19,+21,+22,-X	Abnormal
		9	1	9	46,XY,-5p,-18p	Abnormal
		10	2	10	No result	Undiagnosed
		11	2	9	46,XX	Normal
		12	1	8	45,XX,-16	Abnormal
		13	2	6	46,XYY,+1,+2,+3,-5q,-6,+8,-9,-10,-11,-12,-13,-15,+16,-18,-19,+20,+21,+22	Abnormal
		14	2	8	No result	Undiagnosed
		15	2	11	52,YY,+1,+2,+6,+9,-13,+14,-16,+17,+18,-20,+21,+22,-X	Abnormal
		16	1	10	46,XX	Normal
9	34	1	1	10	46,XX	Normal
		2	1	8	46,XY	Normal
		3	4	6	50,XX,+4,+12,+16,+20	Abnormal
10	35	1	2	7	46,XX	Normal
		2	2	7	48,XY,+14,+22	Abnormal
		3	1	7	46,XX,-7q	Abnormal
		4	1	7	47,XX,+13	Abnormal
11	35	1	2	7	42,XX,-2,-8,-11,-20	Abnormal
		2	2	8	46,XX	Normal
12	35	1	1	8	47,XX,+22	Abnormal
		2	1	8	47,XY,+22	Abnormal
		3	1	8	46,XX	Normal
		4	2	8	48,XY,+11,+22	Abnormal
		5	1	8	46,XX	Normal
		6	2	8	45,XX,-19	Abnormal
13	36	1	2	8	No result	Undiagnosed
		2	2	7	46,XX,-2,+3,+11,-12	Abnormal
14	36	1	2	8	45,XX,-8,+13,-22	Abnormal
		2	1	8	46,XY,-19p	Abnormal
		3	1	8	45,XX,-19	Abnormal
		4	2	8	46,XX,+6p,-6q	Abnormal
		5	2	8	45,XY,-14,-16q	Abnormal
		6	1	9	46,XY	Normal

		7	1	8	46,X0,-12,+13,+21	Abnormal
		8	1	8	46,XY	Normal
		9	1	7	46,XX	Normal
		10	1	7	48,XXY,+22	Abnormal
15	37	1	2	8	46,XX,-7q11.21-qter	Abnormal
16	37	1	4	6	45,XX,-16	Abnormal
		2	4	8	45,XY,-13	Abnormal
		3	2	8	46,XY	Normal
		4	2	9	47,XY,-9q,+14	Abnormal
		5	4	6	45,XY,-8	Abnormal
17	37	1	4	8	50,XY,+1,+7,+12,+18	Abnormal
		2	2	7	45,XY,+7q,-10,-22	Abnormal
		3	4	6	47,XY,+8	Abnormal
		4	1	8	44,XY,-1,-3,-7,+20	Abnormal
		5	2	7	No result	Undiagnosed
		6	3	8	46,XX,+12p,-12q	Abnormal
		7	2	8	44,XX,-19,-22	Abnormal
18	37	1	1	8	45,XX,-15	Abnormal
		2	1	8	45,XX,-20	Abnormal
		3	2	6	48,XY,+11,+17	Abnormal
		4	1	8	45,XY,-13	Abnormal
		5	1	10	44,X,+1,-2,+3,-4,+5,-6,-7,-8,-9,+10,+11,+12,+13,+14,-15,-16,-17,-18,+19,-20,+21,-22	Abnormal
		6	1	9	45,XX,+2,-6,+9,-10,-12	Abnormal
		7	1	8	45,XX,-11	Abnormal
		8	2	6	46,XY	Normal
19	37	1	3	7	47,XY,+16	Abnormal
		2	3	6	47,XX,+7	Abnormal
		3	2	7	46,XX	Normal
		4	3	7	43,XY,-1,-6,+14,-15,+17,-21,-22	Abnormal
		5	2	8	47,XX,+6	Abnormal
		6	3	8	46,XY,+15,-17	Abnormal
		7	3	2	48,XXY,+19	Abnormal
		8	3	8	45,XX,-19	Abnormal

		9	3	8	47,XXY,-1,-13	Abnormal
		10	3	5	47,XY,+22	Abnormal
		11	4	8	47,XX,+8	Abnormal
		12	3	8	46,XY,+9q,-14,-16,+22	Abnormal
		13	3	9	39,X0,-12,-13,-16,-17,-19,-21	Abnormal
		14	3	7	41,XX,+1p,-3,-9,-13,-17,-22	Abnormal
		15	2	8	44,XY,-8,-16	Abnormal
		16	3	7	45,XX,-13	Abnormal
		17	3	10	46,XX,+14,-15	Abnormal
		18	3	9	47,XY,+22	Abnormal
		19	4	8	48,XX,+15,+21	Abnormal
		20	3	8	46,XY,-1,+3,-5,-10,+11,+14	Abnormal
20	38	1	2	7	50,XX,+4,+5,+11,+12	Abnormal
		2	2	11	46,XX	Normal
		3	2	7	45,XX,-14,-16,+22	Abnormal
		4	2	7	No result	Undiagnosed
		5	2	7	45,XX,-11	Abnormal
21	38	1	3	6	36,-2,-3,-10,-11,-17,-18,+19,-20,-21,-22,-X,-Y	Abnormal
		2	3	7-8	44,XX,-4,-9	Abnormal
		3	2	7	No result	Undiagnosed
		4	2	7	44,X0,-4,+13,-22	Abnormal
		5	1	8	44,XY,-8,-11	Abnormal
		6	2	10	49,Y0,+2,-3,+5,-7,+8,-10,+11,-12,+14,-15,+17,+18,+19,+22,-X	Abnormal
		7	2	8	46,XX,+8p,-8q	Abnormal
		8	1	9	50,+3,+6,+7,+8,+9,+10,-19,+22,-X,-Y	Abnormal
22	38	1	2	8	47,XX,+22	Abnormal
		2	2	8	47,XY,-1,+10,+11	Abnormal
		3	2	8	46,XY	Normal
		4	2	8	46,XX	Normal
		5	1	8	49,XY,+7,+8,+13	Abnormal
		6	3	7	42,XY,-7,-8,-14,-15	Abnormal
23	39	1	2	7	No result	Undiagnosed
		2	2	7	No result	Undiagnosed
		3	2	6	45,XX,-15	Abnormal

		4	2	7	43,XX,+3,-4,+10q,-16,-18,-20	Abnormal
24	39	1	2	8	46,XY	Normal
		2	2	6	45,XX,-15	Abnormal
		3	3	4	47,XX,+13	Abnormal
		4	2	7	47,Y0,-6,-8,+12,+13,+15,+18,+19,-21,-X	Abnormal
		5	2	6	46,XX,+12q	Abnormal
		6	2	6	No result	Undiagnosed
25	39	1	3	6	48,XX,+2,+4,+5,-6,-7,-8,+9,-10,-11,-13,+15,+16,+17,+20,+22	Abnormal
		2	2	8	46,XY,+16,-18	Abnormal
26	39	1	1	9	46,XX	Normal
		2	3	6	45,XX,-22	Abnormal
		3	1	8	46,XY,+10p,-14,+17	Abnormal
		4	1	8	45,XX,-21	Abnormal
		5	2	10	46,XX	Normal
		6	2	9	46,XY	Normal
		7	1	8	46,XX	Normal
27	39	1	2	8	45,XX,+18p,-22	Abnormal
		2	2	7	46,XY	Normal
		3	2	8	No result	Undiagnosed
		4	3	9	46,XX	Normal
		5	1	8	46,XY	Normal
		6	2	7	No result	Undiagnosed
		7	2	7	No result	Undiagnosed
28	40	1	3	5	46,XY	Normal
		2	3	4	47,XY,+17	Abnormal
		3	3	6	46,XY	Normal
		4	3	8	46,XY	Normal
		5	2	5	No result	Undiagnosed
		6	3	6	47,XX,-3,+5,+6,-7,-9,+19q,+20,+21	Abnormal
		7	3	8	46,XY	Normal
29	40	1	1	8	45,XX,-14	Abnormal
		2	2	7	47,XX,+8,-15,+16	Abnormal
		3	2	13	47,XX,+22	Abnormal
		4	1	8	45,XY,-11	Abnormal

		5	1	8	47,XY,+15	Abnormal
		6	1	7	48,XY,+15,+21	Abnormal
		7	2	8	47,XY,+10	Abnormal
		8	2	7	42,XX,-14,-15,-20,-21	Abnormal
		9	2	8	43,X0,+6,-10,-14,-22	Abnormal
30	40	1	4	8	48,Y0,-2,-5,+12,+17,+18,+22,-X	Abnormal
		2	4	7	49,XX,+1,+2,-4p,+7,+10,-11,-12,+13,-14,-16,+17,+18,-19,+22	Abnormal
31	40	1	4	4	47,XY,-11q,+22	Abnormal
		2	4	5	45,XX,-13	Abnormal
		3	4	5	46,X0,+17	Abnormal
		4	2	9	45,XX,-22	Abnormal
		5	1	7	45,XX,-2,-16q	Abnormal
		6	4	4	46,XY,-8p,+8q	Abnormal
		7	2	10	42,XY,+3,-4,+12,-14,-16,-17,-18,-20	Abnormal
32	40	1	2	8	48,XY,+1,+2q,+9	Abnormal
		2	2	6	No result	Undiagnosed
		3	2	6	46,XX,+13,-15	Abnormal
		4	2	6	47,XX,+13,+19q	Abnormal
		5	3	8	45,XY,-19	Abnormal
		6	3	5	40,XX,-2,+5,-6,-8,-12,-19,-20,-21	Abnormal
		7	3	8	46,XY	Normal
		8	2	8	43,XX,-12,-15,-16	Abnormal
		9	2	10	50,XX,-1,+2,+3,+5,-8,+10,+12,-13,+16.+17,+18,-20,+21,-22	Abnormal
		10	2	6	No result	Undiagnosed
		11	2	8	No result	Undiagnosed
		12	2	5	46,XX	Normal
		13	2	6	46,XX,-4q28.3_q35.2,-13,+21	Abnormal
33	40	1	2	8	49,XX,+14,+15,+21	Abnormal
		2	3	8	47,XX,+22	Abnormal
		3	3	7	46,XY	Normal
		4	3	8	45,XX,-22	Abnormal
		5	3	6	45,XY,-15	Abnormal
		6	2	8	47,XY,+13	Abnormal
		7	2	8	No result	Undiagnosed

		8	3	8	45,XY,-19	Abnormal
		9	3	8	49,Y0,+4,-6,+8,+10,+14,+16,+17,-18,+19,-22,-X	Abnormal
		10	3	8	53,XXY,+1,+3,+5,-8,+9,+12,+14,-15,-16,+18,+19	Abnormal
		11	3	8	45,Y0,-7,+12,-14,+16,-22,-X	Abnormal
		12	3	8	45,XY,+1,+2,-8,-9,+10,+11,+12,+13,-14,-15,+16,-18,-20,-22	Abnormal
		13	3	8	45,XX,-16	Abnormal
		14	3	5	46,XX	Normal
34	40	1	2	6	44,XX,-1,-5,+15,-18	Abnormal
		2	2	6	48,XX,+18,+19	Abnormal
		3	2	6	No result	Undiagnosed
		4	3	6	47,XX,+8,+9,-15	Abnormal
		5	2	8	No result	Undiagnosed
		6	2	4	47,XY,-1,+2,+9,-18,-20,+21,+22	Abnormal
		7	2	6	No result	Undiagnosed
		8	2	7	48,XXY,-3,-4,-5,-8p,+10,+13,-14,+15,+18,-20,+21,	Abnormal
35	40	1	4	8	47,XX,+17	Abnormal
		2	3	11	48,XXY,+3,+8,-18	Abnormal
		3	3	7	45,XX,+17p,-22	Abnormal
		4	3	9	41,X0,-2,-3,-14,-15	Abnormal
		5	3	6	46,XY	Normal
		6	3	8	46,XX	Normal
		7	3	9	46,XY	Normal
		8	3	10	49,XY,+2,+3,+10	Abnormal
		9	4	8	47,XX,+14	Abnormal
		10	4	5	44,XY,-15,-18	Abnormal
36	41	1	2	7	43,XO,+3,-10,-13,-15	Abnormal
		2	2	7	47,XY,+9	Abnormal
		3	2	7	48,XY,+15,+16	Abnormal
37	41	1	2	9	49,X0,+3,+17,+18p/q,+21	Abnormal
		2	2	7	44,XY,+1,-3,-9p,+14,-16p,-17,-21,-22	Abnormal
		3	1	14	44,XY,-2,-5,+19,-20	Abnormal
		4	1	8	46,XX	Normal
		5	1	7	45,XX,-1,-8p,+12q	Abnormal
		6	1	8	46,X0,+1q,+20	Abnormal

		7	2	8	46,XX,-15,+16	Abnormal
38	41	1	3	6	46,XX,+2,+9q,-15,+18,-21	Abnormal
		2	1	8	44,Y0,-16,-X	Abnormal
		3	4	6	47,XY,+1q,-4,-5,-6,+8,-9,-12,+13,+14,+15,+16,-20	Abnormal
		4	2	8	46,XX,-4,+18,-21,+22	Abnormal
		5	1	7	47,XX,+22	Abnormal
39	41	1	3	6	45,XX,+9,-12,-13,+15,+16,-17,+18,-19,-22	Abnormal
		2	2	8	44,X,-6,-10	Abnormal
		3	2	9	47,XX,+13	Abnormal
		4	2	8	51,X0,+5,+7,+14,+16,+20,+22	Abnormal
		5	2	6	41,XX,-1,-5,-13,-14,+15,-21,-22	Abnormal
40	41	1	2	8	45,XX,-2,-7,+8	Abnormal
		2	2	7	43,XY,-10,-15,-19	Abnormal
		3	2	7	46,XY,-5,+12,-15,+19	Abnormal
41	41	1	2	8	48,XX,+10,+16,+17,-19,-21,+22	Abnormal
		2	1	7	43,XY,-2,-5p,-7,+8q,+9,-21,-22	Abnormal
		3	2	8	46,XY,+21,-22	Abnormal
		4	2	7	44,X0,-2q35_2q37,-14	Abnormal
		5	2	8	48,XY,+15,+22	Abnormal
		6	2	7	46,XX,+12p13.33_12q21.33,-16,+21	Abnormal
		7	1	8	45,X0	Abnormal
		8	1	8	45,XX,-19	Abnormal
42	41	1	2	7	No result	Undiagnosed
		2	3	6	No result	Undiagnosed
		3	2	8-10	No result	Undiagnosed
		4	1	7	No result	Undiagnosed
		5	1	7	44,XY,-2,-17	Abnormal
		6	2	8	45,XX,-1,-11q13.2_q25,+14,-22	Abnormal
		7	1	8	45,XX,+9,-15,-16	Abnormal
43	41	1	2	8	47,XX,+19	Abnormal
		2	2	8	48,XX,-1,+2,+6,+7,+8,+9,-12,-15,+20,-21	Abnormal
		3	3	9	46,XY	Normal
		4	2	6	47,XY,+16	Abnormal
		5	2	8	45,XX,-3q,-22	Abnormal

44	41	1	2	9	47,XXY,-12,+19	Abnormal
		2	2	9	47,XY,-1,+2,-3,+6,+7,+12,-15,-19,+22	Abnormal
		3	3	6	49,X0,+5,+6,+7,+9,+10,-12,+13,-15,+16,-17,+18,-22	Abnormal
		4	1	10	45,XXYY,+1,-2,-4,-8,-9,+10,+12,-13,+14,+15,-16,-17,-19,+20	Abnormal
45	41	1	3	8	51,XX,+1,-2,+5,+7,+8,-16,+17,-18,+20,+21,+22	Abnormal
		2	3	6	46,XY,+2,-15,+16,-22	Abnormal
		3	3	5	48,XY,+21,+22	Abnormal
		4	3	5	46,XX,-1,+2,+3,+4,-5,+6,+18,-20,-21,22	Abnormal
		5	2	5	No result	Undiagnosed
46	41	1	1	7	46,XY,-1,-4,+15,+21	Abnormal
		2	1	8	42,X0,-1,-3,-6q,-10,+12,+13,+14,+15,-18,-21	Abnormal
		3	1	7	47,XXY,+9p	Abnormal
		4	2	6	46,XX,+1,-21	Abnormal
47	42	1	2	8	46,XY	Normal
		2	2	10	46,XX	Normal
		3	2	10	46,XX,-12,+13	Abnormal
		4	2	8	47,XX,+15	Abnormal
		5	2	7	45,XY,-10	Abnormal
		6	2	8	45,XY,-22	Abnormal
		7	2	11	50,X ,+4,+11,+14,+18	Abnormal
		8	2	8	46,XX	Normal
		9	2	10	44,XX,-13,-16	Abnormal
48	42	1	3	6	46,XX,+6,-8,-14,+16	Abnormal
		2	3	5	46,XY,-18q	Abnormal
		3	3	3	38,XX,-5,-7,-10,+14,-15,-16,-17,-18,-21,-22	Abnormal
		4	2	6	No result	Undiagnosed
49	42	1	2	7	45,X0	Abnormal
		2	2	6	45,X0	Abnormal
		3	2	7	46,XX,-9p-q	Abnormal
		4	2	7	41,XX,-6,-7,-13,-14,-17,-19,+22	Abnormal
		5	2	7	44,XY,-8,-22	Abnormal
50	42	1	3	8	44,X,-14,-15	Abnormal
		2	3	8	No result	Undiagnosed
		3	3	8	46,XY	Normal

		4	3	6	46,XX,-11,+21	Abnormal
		5	2	6	No result	Undiagnosed
		6	2	5	48,XX,+5,+10	Abnormal
		7	3	4	No result	Undiagnosed
		8	2	4	47,XY,+7,+19p	Abnormal
		9	2	4	48,XX,+1,+2,+8,+9,-10,-11,-12,-14,+17,+21	Abnormal
51	42	1	2	7	45,XX,+11,-15,-19q,-21	Abnormal
		2	2	8	49,XX,+13,+16,+17	Abnormal
		3	2	7	46,XY,+3,-5,-7,+8,-10,+13,+19,-21	Abnormal
		5	2	7	No result	Undiagnosed
		6	2	7	No result	Undiagnosed
		7	2	7	43,XX,-4,-5,-16	Abnormal
		8	2	6	No result	Undiagnosed
		9	1	6	44,XX,+5,-9,-13,-14,+19,-21,+Xq	Abnormal
		10	2	7	48,XY,+4,+21	Abnormal
		11	2	7	44,XY,-7,-22	Abnormal
52	42	1	4	5	45,X0,-5,-6,-8,+12,+16,-17,+19,+22	Abnormal
		2	2	8	46,XX,-5,+14,+15,+19,-21,-22	Abnormal
		3	3	6	48,XY,+2,-4,-9,+13,-14,+15,+16,-19,+20,+21	Abnormal
53	42	1	1	7	46,XX	Normal
		2	1	8	45,XY,-9	Abnormal
		3	3	6	44,XX,-4,-19	Abnormal
		4	1	8	43,XX,-14,-17,-22	Abnormal
		5	2	7	45,XX,-4q,-5,+7	Abnormal
		6	1	8	37,XY,-2,+5,-6,-7,-8,-12,-14,-16,-17,-18,-20	Abnormal
		7	2	7	53,XX,+3,+6,+8,+9,+17,+19,+22	Abnormal
54	42	1	4	8	46,XY	Normal
		2	2	7	44,XX,-7,-9	Abnormal
		3	2	6	45,XY,-21	Abnormal
		4	2	7	45,XX,-22	Abnormal
		5	3	6	43,XY,-17,19,-22	Abnormal
55	42	1	2	8	46,XY,-Xq	Abnormal
		2	2	9	47,XX,+20	Abnormal
		3	1	8	46,XX,-14,+21	Abnormal

		4	2	8	44,XX,-9,-13	Abnormal
		5	2	9	46,XY,+10,-16	Abnormal
		6	2	7	45,XY,-19	Abnormal
56	42	1	2	5	46,XY,-1,+11,-16,+20,-21,+22	Abnormal
		2	2	10	41,XX,+4,-7,-10,-11,-15,-18,-21	Abnormal
		3	2	10	43,XX,-13,-14,-15	Abnormal
		4	2	5	No result	Undiagnosed
57	42	1	2	8	45,XY,-1	Abnormal
		2	3	11	44,XX,-9,-15	Abnormal
58	43	1	4	7	43,XY,-1,-2,+5,-15,-22	Abnormal
		2	4	6	No result	Undiagnosed
		3	3	8	49,XY,+11,+14,+15	Abnormal
		4	4	8	46,XX,-7,+12,+21,-22	Abnormal
		5	3	8	47,XY,+15,-46,+18	Abnormal
59	43	1	2	8	48.XYY,+6,+7,-8,-9,+13,+14,-15,-16,+20	Abnormal
		2	2	10	49,XXY,+7,-10,+17,-18,+21,+22	Abnormal
		3	2	7	48,+1,-2,+6,+7,+10,+14,+16,-18,+21,-22,-X,-Y	Abnormal
		4	2	7	45,XX,+1,+2,+5,-7,+8,-9,-10,+11,-13,-15,-18,-19,+21,-22	Abnormal
60	43	1	1	8	47,XX,-2,+16,+17,+21,-22	Abnormal
		2	2	8	47,XX,+21	Abnormal
		3	2	10	49,XX,+4,+9,+15	Abnormal
		4	2	9	45,XX,-15,-16,+19	Abnormal
61	43	1	3	8	46,XY	Normal
		3	3	6	46,XY,-5,+9,+15,+17,+19q,-20,-22	Abnormal
		4	2	7	46,XX	Normal
		5	3	5	47,XX,-7p22.3_7q11.21,+7q11.21_7q36.3,+20	Abnormal
62	44	1	2	9	45,XX,-2,-3.-4.+5,-7,+9,+12,+13,+14,-15,-16,-17,-18,-19,+20,+21,-22	Abnormal
		2	2	6	39,X0,-5,-11,-17,-18,-21,-22	Abnormal
		3	2	3-4	46,XX	Normal
		4	2	4	46,XX,-3q	Abnormal
		5	2	7	44,XX,-4,-10,-12p,+12q	Abnormal
		6	1	8	41,-1,-4,+5,-6,+7,-8,-17,-19,-20,+21,-X,-Y	Abnormal
		7	2	4	47,XX,+16	Abnormal

63	44	1	4	10	47,XX,+13	Abnormal
		2	3	6	47,XX,+13	Abnormal
		3	3	10	No result	Undiagnosed
64	44	1	2	6	46,XY	Normal
		2	1	8	48,XY,+16,+22	Abnormal
		3	2	5	48,XX,+13,+17	Abnormal
		4	2	8	47,XX,+1,+14,+15	Abnormal
		5	2	6	45,XY,-11	Abnormal
		6	2	5	44,XX,-9,-13,+16,-20	Abnormal
		7	1	8	47,XY,-4,+12,+16,+21,-22	Abnormal
65	44	1	1	6	45,X0,+2,-17,+18p,+19,-20	Abnormal
		2	1	8	44,XYY,-2,-3,-7,-9,+12,+14,+15,-16,-17,-18,+22	Abnormal
		3	1	7	42,XX,-8,-15,-16,-18	Abnormal
		4	1	7	47,XX,+13,+19p	Abnormal
		5	2	4	46,XY,+2q,+13,-22	Abnormal
		6	1	8	48,XX,-10,+15,+19,+22	Abnormal
66	44	1	1	9	42,XY,-14,-15,-20,-21	Abnormal
		2	1	9	42,XX,-7,-8,-9,-15,+18,-22	Abnormal
		3	2	6	44,X0,-16	Abnormal
		4	1	6	45,XY,+5,-13,+14,-16,-19	Abnormal
		5	1	7	51,XXY,+1,+3,+5,-8,-9,-10,+11,+13,-14,+15,+16,+18	Abnormal
		6	1	9	48,XY,+9,+10	Abnormal
		7	1	8	45,X0	Abnormal
		8	1	8	43,XX,-7,-15,-21	Abnormal
		9	1	5	46,XY	Normal
67	45	1	3	10	50,XX,+5,-9,+11,+13,+19,+22	Abnormal
		2	3	10	45,XY,+12,-16,-21	Abnormal
		3	3	8	45,XX,-16	Abnormal
		4	3	5	46,XX	Normal
		5	3	7	42,XY,-3,-4,-17,-18,+19,-20	Abnormal
		6	3	5	49,XY,+1,-2,-3,+5,-6,+7,+8,-9,+10p,-11,+12,+14,+15,part16,+17,+18,+19q,-20,+21,-22	Abnormal
		7	4	4	46,X,-6,-9p,+21,-Xq	Abnormal
		8	3	5	47,XY,+13,-14,+19	Abnormal

Table B-02: Patient details and blastocyst stage embryo morphology and karyotypes

Patient	Age	Embryo	Expansion Grade	ICM grade	TE grade	CGH result	Embryo characterisation
1	29	1	2/3			46,XX	Normal
		2	5	A	B	46,XX	Normal
		3	5	B	B	47,XY,+16	Abnormal
		4	5	A	A	46,XY	Normal
		5	2/3			46,XX,+1p36.3-p11	Abnormal
		6	4	A	B	46,XX	Normal
		7	5	B	B	47,XY,+18	Abnormal
2	30	1	3	A	B	47,XXY	Abnormal
		2	4	A	A	46,XY	Normal
3	30	1	3			47,XX,+19	Abnormal
		2	3	B	A	46,XY	Normal
		3	3			45,XO	Abnormal
		4	3	A	A	46,XY	Normal
4	30	1	4	A	A	46,XX	Normal
		2	4	B	A	No result	Undiagnosed
		3	3	A	A	No result	Undiagnosed
		4	3	A	A	No result	Undiagnosed
		5	4	B	B	46,XY	Normal
5	30	1	4	A	B	46,XY	Normal
		2	4	B	B	47,XY,+18	Abnormal
		3	4	B	B	No result	Undiagnosed
		4	3	B	B	47,XY,+4	Abnormal
6	30	1	3	A	A	46,XX	Normal
		2	4	B	B	46,XY	Normal
		3	3	A	A	45,XX,-19	Abnormal
		4	3	A	B	46,XY,-18,+22	Abnormal
		5	2	B	C	40,XY,-2,+3,+4,+5,-6,-7,+9,-10,+11,-12,+13,+15,-17,+19,-20	Abnormal
		6	2			46,XY	Normal
		7	3	B	B	46,XY	Normal
		8	4	A	A	45,X0	Abnormal
		9	3	A	B	45,XX,-5	Abnormal

		10	3	A	A	46,XX	Normal
		11	4	A	A	46,XY	Normal
		12	3	B	B	46,XX	Normal
		13	4	A	A	46,XY	Normal
7	30	1	2	D	D	46,XX	Normal
		2	2	D	D	45,XY,-2	Abnormal
		3	3	C	C	45,XX,-15	Abnormal
		4	3	B	C	46,XX	Normal
		5	5	B	B	46,XX	Normal
		6	3	C	C	46,XY	Normal
		7	3	B	B	46,XX	Normal
		8	3	D	C	45,XX,-21	Abnormal
		9	3	D	D	46,XY	Normal
8	31	1	3	C	C	45,XX,-15	Abnormal
		2	3	C	C	46,XX	Normal
		3	3/4	C	C	45,XX,-4	Abnormal
		4	3	C	C	46,XX	Normal
		5	3	B	C	46,XY	Normal
		6	3/4	C	C	46,XX,-13,+15	Abnormal
		7	3	B	C	No result	Undiagnosed
		8	3	C	C	46,XX	Normal
		9	3	B	C	46,XX	Normal
		10	3	C	C	47,XY,+2	Abnormal
9	31	1	5	A	B	45,XX,-3q13.31_3q29,-19	Abnormal
		2	5	A	B	No result	Undiagnosed
		3	4	B	B	46,XX	Normal
		4	3	B	B	No result	Undiagnosed
		5	3	B	B	46,XY	Normal
		6	4	B	B	46,XY	Normal
		7	3	B	B	46,XX	Normal
		8	3	B	B	46,XY	Normal
10	32	1	4	A	A	47,XY,+19	Abnormal
		2	3	B	B	46,XX	Normal
		3	4	A	A	46,XY	Normal

		4	3	B	C	46,XX	Normal
		5	4	A	A	47,XXY	Abnormal
		6	3	B	C	47,XXY	Abnormal
		7	3	B	B	46,XO,-4,+5q23.3-q35.2,-6,+15,+22	Abnormal
		8	3	B	B	46,XY	Normal
		9	2/3			46,XY	Normal
		10	4	B	B	No result	Undiagnosed
11	32	1	5	B	A	46,XX	Normal
		2	5	B	B	47,XY,+15	Abnormal
		3	2/3			46,XX	Normal
		4	4	B	B	46,XY	Normal
		5	2/3			46,XY,-15,+19	Abnormal
		6	4	B	B	45,XX,-14	Abnormal
		7	5	B	A	46,XX	Normal
		8	4	B	A	46,XY	Normal
		9	4	A	B	46,XX	Normal
12	32	1	4	B	B	46,XY	Normal
		2	4	A	A	46,XY	Normal
		3	3	B	B	46,XX	Normal
		4	4	A	A	No result	Undiagnosed
		5	2/3			47,XX,+4	Abnormal
		6	4	B	C	47,XX,+15,+2p25.2-2q12.1	Abnormal
		7	4	A	B	46,XX	Normal
		8	5	B	B	45,XY,-16	Abnormal
		9	4	A	B	46,XX	Normal
13	32	1	4	A	B	47,XY,+22	Abnormal
		2	4	A	B	No result	Undiagnosed
		3	3	A	A	45,XY,-6q24-qter	Abnormal
		4	3	A	A	46,XY	Normal
		5	6	A	A	46,XY	Normal
		6	5	B	B	46,XX	Normal
14	33	1	4	A	B	46,XY	Normal
		2	4	B	A	46,XY	Normal
		3	3	A	A	46,XX	Normal

		4	4	B	B	No result	Undiagnosed
		5	3	A	A	46,XX	Normal
		6	5	A	A	No result	Undiagnosed
15	33	1	5	A	A	46,XX	Normal
		2	3	B	B	47,XY,+22	Abnormal
		3	3	B	B	45,XX,-2p25.3-q32.1,+2q32.2-q37.3,-10q11.2-q26.3	Abnormal
		4	4	B	B	48,XX,+19,+22	Abnormal
		5	4	B	B	47,XY,+22	Abnormal
		6	4	A	B	46,XY,-1q32.1-q44	Abnormal
16	33	1	2/3			42,XO,-9,-11,-13	Abnormal
		2	4	A	A	46,XY,	Normal
		3	5	A	A	48,XXYY	Abnormal
		4	4	B	B	46,XY	Normal
		5	4	A	A	46,XX	Normal
17	33	1	3	A	B	46,XX	Normal
		2	3	B	B	46,XX	Normal
		3	3	B	B	45,XO	Abnormal
		4	4	B	A	47,XY,+1	Abnormal
		5	4	A	A	46,XY	Normal
18	33	1	3	A	B	45,XY,-8	Abnormal
		2	3	A	A	45,XY,-2	Abnormal
		3	4	B	B	45,XY,-16	Abnormal
		4	3	B	A	45,XX,-1	Abnormal
		5	5	A	B	46,XY	Normal
		6	3	B	B	46,XX	Normal
		7	4	B	A	46,XY,-4p16-p11,+4q11-q35	Abnormal
		8	4	A	A	47,XX,-3q11.2-q29,+15	Abnormal
		9	4	A	A	46,XY	Normal
		10	4	B	B	48,XX,+7,+22	Abnormal
		11	5	B	B	48,XX,+8,+10	Abnormal
19	34	1	4	B	B	45,XX,-4	Abnormal
		2	4	B	B	47,XX,+22	Abnormal
		3	5	A	A	45,XX,-2	Abnormal
		4	4	B	B	47,XX,+5	Abnormal

		5	4	A	B	46,XX	Normal
		6	4	B	C	45,XY,-13	Abnormal
		7	4	A	B	46,XX	Normal
		8	3	A	B	46,XX	Normal
		9	6	A	A	46,XY	Normal
20	34	1	3	A	A	No result	Undiagnosed
		2	5	A	A	45,XX,-21	Abnormal
		3	3	B	A	No result	Undiagnosed
		4	3	B	A	No result	Undiagnosed
		5	4	A	A	47,XX,+22	Abnormal
		6	4	B	A	No result	Undiagnosed
		7	3	A	A	47,XX,+22	Abnormal
		8	6	A	A	47,XX,+22	Abnormal
		9	4	A	A	45,XO	Abnormal
		10	5	A	A	46,XX,-5,+22	Abnormal
21	34	1	3	B	A	46,XY	Normal
		2	3	B	A	46,XX	Normal
		3	4	B	B	46,XX	Normal
		4	3	B	A	47,XY,+22	Abnormal
		5	3	A	B	46,XX,-10q11.2-q26.3	Abnormal
		6	3	A	A	46,XX	Normal
		7	3	B	A	46,XX	Normal
22	34	1	3	B	B	46,XX	Normal
		2	4	B	A	47,XY,+22	Abnormal
		3	4	B	B	46,XO,+22	Abnormal
		4	3	B	B	46,XY	Normal
		5	4	A	B	46,XY	Normal
		6	4	B	B	46,XY	Normal
		7	4	A	B	No result	Undiagnosed
		8	4	A	A	46,XX	Abnormal
23	34	1	4	A	A	47,XX,+19	Abnormal
		2	4	B	B	47,XX,+19	Abnormal
		3	3	B	B	45,XX,-12	Abnormal
		4	3	B	B	47,XX,+17	Abnormal

24	34	1	3	A	B	46,XX	Normal
		2	3	B	C	46,XX	Normal
		3	3	A	A	46,XX	Normal
		4	3			46,XX,-13,+16	Abnormal
		5	3	A	A	47,XXY	Abnormal
		6	2/3			46,XY,+16pter-p11.1	Abnormal
		7	4	B	A	46,XX	Normal
		8	4	B	B	46,XX	Normal
		9	3	B	A	46,XX	Normal
		10	2/3			45,XX,-11	Abnormal
		11	2/3			46,XY	Normal
		12	6	B	A	46,XX	Normal
		13	5	B	B	46,XY	Normal
		14	6	B	A	46,XX	Normal
		15	4	B	B	46,XX	Normal
		16	3	B	A	46,XY	Normal
25	34	1	3	B	B	46,XX	Normal
		2	3	B	B	No result	Undiagnosed
26	34	1	4	A	A	SG failure/ No result	Undiagnosed
		2	4	B	A	SG failure/ No result	Undiagnosed
		3	3	A	A	SG failure/ No result	Undiagnosed
		4	2/3			46,XX,-2q11.2-q37.3	Abnormal
		5	3	B	B	46,XY,-15,+19	Abnormal
		6	3	B	B	45,XO	Abnormal
		7	4	B	B	47,XY,+16	Abnormal
		8	4	A	A	47,XX,+8	Abnormal
		9	4	A	A	46,XY	Normal
		10	4	A	A	46,XY	Normal
		11	2/3			47,XX,+19	Abnormal
		12	4	A	A	46,XY,-7q11.2-q36	Abnormal
		13	4	A	A	46,XX	Normal
		14	4	A	B	46,XY	Normal
		15	3	A	B	46,XX	Normal
27	34	1	4	B	B	45,XX,-16	Abnormal

		2	2/3			46,XX	Normal
		3	5	B	B	No result	Undiagnosed
		4	5	B	B	46,XX	Normal
28	34	1	4	A	A	46,XY	Normal
		2	6	A	A	46,XY	Normal
		3	5	A	A	46,XY	Normal
		4	4	A	A	46,XX,-22	Abnormal
		5	4	A	A	46,XY,+22	Abnormal
		6	4	A	B	46,XX	Normal
		7	4	A	A	46,XX	Normal
		8	4	A	B	46,XX	Normal
		9	2/3			No result	Undiagnosed
29	35	1	3	B	B	46,XX	Normal
		2	3	C	B	46,XY	Normal
		3	3	C	C	46,XX	Normal
		4	3	B	B	46,XY	Normal
30	35	1	4	A	A	46,XX	Normal
		2	5	A	A	No result	Undiagnosed
		3	2/3			46,XY	Normal
		4	4	A	B	46,XY	Normal
		5	5	A	A	No result	Undiagnosed
31	35	1	4	A	B	46,XY	Normal
		2	3	B	C	47,XX,+13	Abnormal
		3	3	B	C	46,XY	Normal
		4	3	B	B	47,XY,+16	Abnormal
		5	2	B	B	46,XY	Normal
32	35	1	4	A	B	45,XY,-18	Abnormal
		2	5	B	B	45,XY,-16	Abnormal
		3	4	B	B	46,XX	Normal
		4	3	A	B	45,XX,-16	Abnormal
		5	3	B	B	SG failure/ No result	Undiagnosed
		6	5	A	A	46,XX,+1q21.1-q44	Abnormal
33	35	1	4	A	A	46,XX	Normal
		2	5	A	A	46,XY	Normal

		3	5	B	A	46,XX	Normal
		4	3	A	A	46,XX	Normal
		5	3	B	B	44,-X,-Y	Abnormal
		6	3	A	B	45,Y0	Abnormal
		7	5	A	B	46,XY	Normal
		8	3	B	B	45,Y0	Abnormal
34	36	1	2	B	B	47,XX,+22	Abnormal
		2	3	B	B	46,XY	Normal
		3	3	B	B	46,XY	Normal
		4	3	B	B	47,XY,+15,-19p13.3-q13.13	Abnormal
35	36	1	4	B	A	46,XY	Normal
		2	4	A	B	45,XX,-13	Abnormal
		3	4	B	A	46,XX	Normal
		4	3	A	B	46,XX	Normal
		5	3	A	A	46,XY	Normal
		6	4	A	A	46,XY	Normal
		7	4	A	A	46,XY	Normal
		8	4	A	A	46,XX,+16	Abnormal
		9	4	A	A	46,XO,+20	Abnormal
		10	4	B	B	45,XO,-11q23.2-qter	Abnormal
		11	3			45,XX,-15	Abnormal
36	36	1	2/3			46,XX	Normal
		2	2/3			46,XX	Normal
		3	4	B	B	46,XY	Normal
		4	3	A	B	46,XX	Normal
37	36	1	5	A	B	45,X0	Abnormal
		2	5	B	B	47,XY,+16	Abnormal
		3	5	B	B	46,XY	Normal
		4	4	B	B	46,XY,-7q11.22-qter	Abnormal
		5	5	C	B	47,XX,+15	Abnormal
		6	6	B	B	46,XY,-5pter-5p12	Abnormal
	cycle 2	7	4	A	A	46,XX	Normal
		8	4	B	A	45,XY,-13	Abnormal
		9	3	A	A	45,XX,-4,+15,-21	Abnormal

		10	3	A	B	47,XY,+12	Abnormal
		11	5	A	A	47,XY,+22	Abnormal
		12	3	B	B	No result	Undiagnosed
38	36	1	5	B	B	47,XY,-4,+12,+22	Abnormal
		2	4	C	B	45,XX,-18,+19,-20	Abnormal
		3	4	B	C	46,XX	Normal
		4	4	B	C	46,XX,-4,+9	Abnormal
		5	3	B	B	47,XX,-4,+20,-21	Abnormal
		6	4	B	B	47,XX,+16	Abnormal
		7	3	B	C	No result	Undiagnosed
		8	3	B	B	46,XY,-6q24.1-qter	Abnormal
		9	3	B	A	46,XX	Normal
39	36	1	4	A	A	46,XY	Normal
		2	4	B	B	48,XX,-3,+12,+13,+18	Abnormal
		3	4	B	B	45,XX,-19	Abnormal
		4	4	A	A	47,XY,+1	Abnormal
40	36	1	4	A	A	45,Y,-X	Abnormal
		2	4	B	A	46,XY	Normal
		3	4	B	B	46,XX	Normal
		4	4	B	B	46,XY,-2p,+2q,+7,+20,+21	Abnormal
		5	4	C	B	No result	Undiagnosed
		6	4	C	B	No result	Undiagnosed
		7	2/3			46,XX	Normal
		8	4	B	B	46,XX	Normal
		9	4	B	C	47,XXY,-3,+4,+5,-6,-7,+10,-13,+14,+15,-17,+18	Abnormal
41	36	1	3	A	A	45,XX,-16	Abnormal
		2	3	A	A	45,XX,-16	Abnormal
		3	3	B	A	46,XY	Normal
		4	2/3			46,XX,-Xp22.2-p21.1	Abnormal
		5	4	A	A	46,XX	Normal
		6	4	A	A	46,XX	Normal
		7	3	A	A	46,XY	Normal
		8	2/3			45,Y0,-X	Abnormal
		9	3	A	B	46,XX	Normal

		10	5	A	A	45,XX,-4	Abnormal
		11	5	A	B	46,XY	Normal
		12	4	A	B	44,XX,-16,-22	Abnormal
		13	4	A	B	45,XY,-22	Abnormal
		14	3	B	B	45,XXY,-16,-21	Abnormal
		15	2/3			46,XY,-1,+22	Abnormal
42	36	1	3	B	B	46,XX,-2,+7	Abormal
		2	3			46,XX	Normal
		3	4	A	B	46,XX	Normal
		4	5	A	A	47,XX,+16	Abnormal
		5	4	A	B	46,XX	Normal
43	36	1	5	B	B	46,XY	Normal
44	36	1	5	A	A	46,XX	Normal
		2	4	A	A	45,XY,-22	Abnormal
		3	3	A	A	46,XY	Normal
		4	4	A	A	46,XX	Normal
		5	3	B	B	46,XX	Normal
		6	3	B	B	No result	Undiagnosed
45	37	1	3	A	A	No result	
		2	3	A	A	No result	
		3	3	A	B	No result	
		4	4	A	A	No result	
		5	3	B	C	46,XX	
		6	3	B	C	No result	
46	37	1	3	A	A	47,XY,+22	Abnormal
		2	4	A	A	43,XX,-8,-13,-18	Abnormal
		3	3	B	B	44,XX,-16,-22	Abnormal
		4	3	A	A	46,XY	Normal
		5	4	A	A	45,XY,-21	Abnormal
		6	3	B	B	48,XXYY	Abnormal
		7	4	A	A	46,XY	Normal
		8	4	B	C	47,XY,+21	Abnormal
		9	4	A	B	46,XX	Normal
		10	3	B	C	47,XX,+21	Abnormal

47	37	1	2/3			46,XY	Normal
		2	3	B	B	44,XX,-+1q21.1-q44,-21,-22	Abnormal
		3	5	A	A	46,XY	Normal
		4	5	A	B	46,XY	Normal
		5	2/3			45,XY,+1q21.1-q44,-2	Abnormal
		6	5	A	A	46,XY	Normal
		7	5	B	A	46,XX	Normal
		8	4	A	B	46,XY	Normal
		9	5	A	A	47,XY,+19	Abnormal
48	37	1	5	A	B	46,XY	Normal
		2	5	A	B	47,XY,+19	Abnormal
		3	4	B	B	45,XX,-4	Abnormal
		4	5	B	B	48,XX,+19,+22	Abnormal
		5	5	A	B	46,XY	Normal
49	37	1	3	A	A	46,XY	Normal
		2	4	A	A	47,XXY	Abnormal
		3	3	B	A	48,XX,+9,+11	Abnormal
		4	5	A	A	46,XY	Normal
		5	3	A	A	47,XY,+17	Abnormal
		6	3	A	B	44,XX,-16,-21	Abnormal
		7	3	A	A	44,XX,-19,-22	Abnormal
		8	3	B	A	46,XX	Normal
50	37	1	5	B	A	45,XO	Abnormal
		2	5	A	B	46,XX	Normal
		3	5	A	C	45,XX,-15	Abnormal
		4	5	B	B	46,XX	Normal
		5	3	A	B	46,XY	Normal
51	37	1	5	B	C	46,XX,+14,-21	Abnormal
		2	6	B	B	46,XY	Normal
		3	5	B	B	46,XY	Normal
		4	5	B	C+	46,XY	Normal
		5	5	C+	B	45,XX,-8	Abnormal
		6	5	A	B	45,XY,-22	Abnormal
		7	5	B	B	47,XX,+22	Abnormal

		8	5	C+	B	46,XX	Normal
		9	5	C+	B	46,XX	Normal
52	37	1	3	B	A	46,XY	Normal
		2	4	A	B	46,XY	Normal
		3	4	B	B	46,XX	Normal
		4	3	A	A	46,XY	Normal
		5	4	A	A	47,XX,+19	Abnormal
		6	4	A	C	48,XY,+13,+16	Abnormal
		7	3	B	B	46,XX	Normal
		8	6	A	A	46,XY,-9,+19	Abnormal
		9	4	B	B	45,XY,-16	Abnormal
53	37	1	3	B	A	47,XY,+22	Abnormal
		2	3			44,XX,-9,-11,-13,+15	Abnormal
		3	4	A	B	46,XX	Normal
		4	5	A	B	46,XX	Normal
54	37	1	3	A	A	46,XX	Normal
		2	3	B	B	No result	Undiagnosed
		3	2/3			45,XX,-5	Abnormal
		4	3	B	A	46,XY	Normal
55	37	1	3	A	B	46,XX	Normal
		2	4	A	B	46,XY	Normal
		3	3	A	B	46,XY	Normal
		4	3	A	B	46,XX	Normal
		5	3	B	B	45,XX,-7	Abnormal
56	37	1	4	B	B	47,XY,+15	Abnormal
		2	4	C	B	46,XY	Normal
		3	2			46,XX,+15,-22	Abnormal
		4	5	B	C	46,XX	Normal
		5	5	B-	B-	48,XX,+1,+5	Abnormal
		6	4	B	C-	46,XX	Normal
57	37	1	2			45,XY,-22	Abnormal
		2	2			46,XY	Normal
		3	2			46,XX	Normal
58	37	1	5	B	B	47,XY,+16	Abnormal

		2	5	B	B	47,XY,+16	Abnormal
		3	5	B	B	47,XX,+20	Abnormal
59	38	1	4	B	B	47,XYY	Abnormal
		2	5	A	A	46,XY	Normal
		3	3	B	B	46,XX	Normal
		4	4	A	A	46,XY	Normal
		5	4	A	B	46,XY	Normal
		6	3	B	B	SG failure/ No result	Undiagnosed
60	38	1	4	A	B	46,XX	Normal
		2	4	B	B	45,XY,-2	Abnormal
		3	4	A	A	46,XY	Normal
		4	3	A	A	45,XO	Abnormal
		5	4	A	A	46,XY	Normal
		6	4	A	B	45,XY,-22	Abnormal
		7	4	A	A	SG failure/ No result	Undiagnosed
		8	3	A	A	SG failure/ No result	Undiagnosed
		9	5	A	A	SG failure/ No result	Undiagnosed
		10	5	A	A	SG failure/ No result	Undiagnosed
		11	5	A	A	46,XX	Normal
		12	4	A	A	44,XX,-18,-22	Abnormal
61	38	1	5	A	A	47,XX,+22	Abnormal
		2	3	A	B	46,XX	Normal
		3	4	B	B	45,XY,-13	Abnormal
62	38	1	2/3			45,XX,-11	Abnormal
		2	4	B	B	46,XY	Normal
		3	5	B	A	46,XX	Normal
		4	4	B	A	45,XY,-11	Abnormal
		5	4	A	A	46,XY	Normal
63	38	1	3	A	A	46,XY	Normal
		2	4	A	A	46,XX	Normal
		3	3	A	B	45,XX,-21	Abnormal
		4	4	B	B	45,XX,-13	Abnormal
		5	2/3			45,XX,-1,-2,+8,-10,+11,+14,-15,-16,+17,+18,-20	Abnormal
		6	2/3			46,XY	Normal

64	38	1	3	C	C	46,XY	Normal
		2	3	C	C	44,XY,+3,-4,+7,-9,-17,-18	Abnormal
		3	2			44,XY,-5q12-5qter,-17,-21,-22	Abnormal
		4	2			44,XY,-9,-16	Abnormal
		5	3	A	C	46,XX	Normal
		6	1			47,XY,+8	Abnormal
65	38	1	3	B	B	49,XY,+3,+21,+22	Abnormal
		2	3	A	C	46,XX,-2p25.3_2p16.3,-8q11.1_q24.3,-10p15.3_p11.21,-12p13.3_12p12.2,+14q23.3_q32.3,-22q13.2_q13.33	Abnormal
		3	5	A	C	47,XX,+6	Abnormal
66	38	1	4	B	b	46,XX	Normal
		2	1			46,XX,+1q31.2_q44,-2q22.3_q37.3	Abnormal
		3	1			42,XX,-1q31.2_q44,+2q22.3_q37.3,-3,-4,-17,-22	Abnormal
		4	3/4	C	b	46,XY	Normal
		5	2			46,XX	Normal
		6	4	B	C	46,XX,-21,+22	Abnormal
67	38	1	2			46,XX	Normal
		2	1			47,XY,+10	Abnormal
68	38	1	3			46,XX,-6,+10,-14,+17,-19,+22	Abnormal
		2	4	A	B	46,XX,-15,+22	Abnormal
		3	5	B	A	47,XY,+22	Abnormal
		4	3			45,XY,-11PTER-P11,-19	Abnormal
69	39	1	4	A	B	No result	Undiagnosed
		2	3	B	A	No result	Undiagnosed
		3	3			No result	Undiagnosed
		4	4	A	B	45,XX,-21	Abnormal
		5	4	A	B	46,XY	Normal
70	39	1	3	B	B	46,XY	Normal
		2	3	B	B	46,XY,-18q	Abnormal
		3	4	B	B	47,XX,+22	Abnormal
71	39	1	2			No result	Undiagnosed
		2	3	B	B-	No result	Undiagnosed
		3	3	B	C	No result	Undiagnosed
		4	3	C	C	No result	Undiagnosed

		5	3	B	C	45,XY,-22	Abnormal
		6	3	C	C	No result	Undiagnosed
		7	3	C	C	No result	Undiagnosed
		8	3	C	C	No result	Undiagnosed
		9	3	B	B	No result	Undiagnosed
		10	3	B	B-	46,XY	Normal
		11	3	B	C-	No result	Undiagnosed
		12	3	C+	C+	No result	Undiagnosed
		13	6	B	B	46,XY	Normal
		14	3	B	C+	47,XX,+15	Abnormal
		15	3	C	C	45,XY,-21	Abnormal
72	39	1	2/3			45,XY,-14	Abnormal
73	39	1	4	B	B	46,XY	Normal
		2	4	B	B	46,XX	Normal
		3	4	A	B	46,XX	Normal
		4	4	B	A	46,XY	Normal
		5	4	A	A	46,XY44,XY,-15,-18	Abnormal
		6	3	A	B	46,XX,-6,+17	Abnormal
		7	4	B	B	47,XY,-19,+20,+2-	Abnormal
		8	4	A	A	46,XYY,-21	Abnormal
		9	4	A	A	46,XY	Normal
		10	3	A	B	46,XX	Normal
		11	3	A	B	46,XX	Normal
74	39	1	4	B	B	46,XY	Normal
		2	4	B	A	46,XY	Normal
		3	3	B	C	46,XYY	Abnormal
75	39	1	3	A	B	46,XX	Normal
		2	3			46,XX	Normal
		3	3	A	A	46,XY	Normal
		4	6	THAWED		46,XY	Normal
		5	3	THAWED		45,XX,-2,+14,-17	Abnormal
		6	3	A	A	47,XX,+18	Abnormal
		7	5	A	B	46,XX	Normal
		8	3	A	B	47,XX,+6,+17,-21	Abnormal

		9	4	A	A	47,XX,+16	Abnormal
		10	3	B	B	45,XY,-21	Abnormal
		11	2/3			44,XX,-13,-16	Abnormal
		12	3	B	B	46,XY	Normal
76	39	1	3	B	B	47,XY,+22	Abnormal
		2	3	B	C	46,XX,-16,+21	Abnormal
		3	2/3	C	C	No result	Undiagnosed
		4	3	C	C	46,XX	Normal
		5	3	C	C	46,XX	Normal
		6	3	C	C	47,XY,+6	Abnormal
		7	1			47,XY,+13	Abnormal
		8	3	c	c	46,XX	Normal
77	39	4	4	B	A	46,XX	Normal
		5	3	B	A	47,XY,+17	Abnormal
		9	4	A	A	46,XX	Normal
		11	3	B	B	47,XX,-11,+12,+22	Abnormal
		12	4	B	B	46,XY	Normal
		13	4	A	B	46,XY	Normal
		14	4	A	A	46,XX	Normal
		15	3	B	B	45,XX,-18	Abnormal
		16	3	B	A	44,X0,-5	Abnormal
		17	4	A	A	46,XY	Normal
		18	3	B	B	46,XY	Normal
		19	3	B	A	45,XX,-16	Abnormal
		20	4	A	A	46,XX,-9,+22	Abnormal
		21	3	B	B	47,XY,+2	Abnormal
78	39	1	3	B	B	47,XY,-4,+21,+22	Abnormal
		2	4	A	B	47,XY,-4q(entire arm),+22	Abnormal
		4	3	A	A	46,XX	Normal
		6	3	B	B	46,XX	Normal
		11	4	B	B	No result	Undiagnosed
		C2_4	3	A	C	45,XX,-22	Abnormal
		C2_10	2/3			46,XX	Normal
		C2_11	4	B	C	46,XY	Normal

79	39	2	3	B	B	46,XX	Normal
		6	3	B	A	46,XX	Normal
		7	4	B	B	47,XY,+22	Abnormal
		10	3	B	B	47,XY,+13	Abnormal
		12	5	B	A	45,XY,-20	Abnormal
		14	3	B	B	46,XY,-14,+22	Abnormal
		15	4	B	B	47,XX,+16	Abnormal
		21	2/3			47,XX,+16	Abnormal
		C2_1	3	A	B	46,XX	Normal
		C2_5	3	B	B	44,XX,-9q33-qter,-15,-16	Abnormal
		C2_7	4	B	A	45,XY,-11,-21,+22	Abnormal
		C2_9	4	A	B	47,XY,+22	Abnormal
		C2_11	5	A	A	47,XY,+14	Abnormal
		C2_13	4	B	C	47,XX,+15	Abnormal
		C3_1	4	A	A	46,XX	Normal
		C3_2	3	B	A	44,XY,-4,-13	Abnormal
		C3_3	5	B	B	46,XY	Normal
80	39	1	2	B	B	47,XY,+18	Abnormal
81	39	1	5	A	A	No result	Undiagnosed
		2	5	B	B	44,X0,-20	Abnormal
82	39	3	6	A	C	46,XY	Normal
83	40	1	3	A	C	46,XY	Normal
84	40	1*	3			46,XX	Normal
		2*	3			46,XY,-1q21.1-q43	Abnormal
		3*	3	A	A	46,XX	Normal
		4*	3			45,XO	Abnormal
		5	4	A	A	47,XX,+15	Abnormal
		6	5	B	B	49,XY,+15,+20,+21	Abnormal
		7	5	C	B	47,XY,+3p26-p11,-3q13.1-q29,+9,-13,+15	Abnormal
		8	2			46,YO,+20,-X	Abnormal
85	40	1	5	A	A	46,XY	Normal
		2	5	A	A	47,XX,+21	Abnormal
86	40	1	5	A	B	46,XX	Normal
		2	6	A	A	45,X0	Abnormal

		9	3	A	B	46,XY	Normal
		10	3	A	B	48,XY,+16,+22	Abnormal
		11	5	B	B	46,XY	Normal
87	40	1	3	C	C	47,XY,+16	Abnormal
		2	3	C	C	46,XY	Normal
		4	3	B	C	47,XX,+20	Abnormal
		7	3	B	C	44,XY,-21,-22	Abnormal
		9	3	B	C	No result	Undiagnosed
		10	1/2			46,XY	Normal
		11	3	B	C	44,XX,-21,-22	Abnormal
		12	3	B	C	46,XY	Normal
88	40	10	3	C	B	44,XX,-13,-21	Abnormal
		12	4	B	B	47,XXY	Abnormal
		14	4	A	B	46,XY	Normal
		C2-5	3			No result	Undiagnosed
		C2-6	3	B	B	45,XX,-21	Abnormal
		C2-9	4	C	B	47,XXY	Abnormal
89	40	1	5	B	A	46,XY	Normal
		2	4	A	A	47,XY,+15	Abnormal
		3	4	B	A	46,XY	Normal
		4	1			45,XY,-22	Abnormal
		5	4	B	B	45,XX,-15	Abnormal
		6	4	A	B	47,XXY	Abnormal
90	40	2	3	B	B	44,X,-7,-21	Abnormal
		3	4	B	B	45,XX,-15	Abnormal
		6	4	A	A	46,XX	Normal
		9	4	B	B	46,XX	Normal
		11	3			46,XX,-Xp22.3-11.23	Abnormal
		12	3	B	A	46,XX	Normal
		13	4	A	A	46,XY	Normal
		14	4	B	B	45,XX,-15	Abnormal
91	40	1	4	A	B	No result	Undiagnosed
		2	4	A	A	46,XX	Normal
		3	3	A	A	No result	Undiagnosed

		4	4	B	C	No result	Undiagnosed
		5	4	A	A	46,XY	Normal
		6	3	A	A	No result	Undiagnosed
		8	4	A	A	46,XY,+20,-21	Abnormal
		16	4	B	A	46,XX	Normal
		22	4	A	B	46,XX	Normal
		24	4	B	A	47,XY,+22	Abnormal
92	40	4	2/3			45,XX,-3	Abnormal
		5	5	A	A	46,XY	Normal
		6	5	A	A	46,XX	Normal
		8	3	A	A	45,XX,-15	Abnormal
		10	4	A	A	46,XY,-21,+22	Abnormal
		14	4	A	A	46,XY,-15,+17	Abnormal
		16	4	A	A	48,XX,+19,+22	Abnormal
		17	3	B	B	45,XX,-12	Abnormal
		18	3	A	A	47,XX,+16	Abnormal
		19	3	A	A	46,XY	Normal
		20	3	A	A	46,XX	Normal
		21	4	B	B	47,XY,-15,+16,+19	Abnormal
		22	3	B	B	46,XX	Normal
		23	3	B	B	46,XX,+Xp22.-p11	
		24	5	B	B	47,XY,+16	Abnormal
93	40	1	4	A	A	45,XY,-22	Abnormal
		3	4	A	A	SG failure/ No result	Undiagnosed
		8	3	B	B	46,XX	Normal
		12	4	B	A	47,XY,+22	Abnormal
		13	3	A	B	46,XY	Normal
		15	3	A	B	46,XX	Normal
		16	4	A	B	46,XY,-15,+21	Abnormal
94	40	1	3	A	A	46,XY,-13,+21	Abnormal
		6	3	A	A	45,XY,-22	Abnormal
		7	5	B	A	46,XY	Normal
		10	4	A	B	47,XX,+9	Abnormal
		11	5	A	A	46,XX	Normal

95	40	2	3	A	B	46,XY	Normal
		10	4	B	B	46,XX,+7,-13	Abnormal
		11	3	A	B	47,XY,+10	Abnormal
		12	4	B	C	46,XX	Normal
		17	4	A	B	43,XX,-5,-18,-21	Abnormal
		19	3	B	C	46,XX	Normal
		21	3	B	C	44,XX,-3.-13	Abnormal
96	40	3	4	B	B	46,XX	Normal
97	40	1	3	B	B	46,XX	Normal
		5	4	B	B	46,XY	Normal
		12	4	A	B	46,XX,+16q11.1-q24	Abnormal
		16	4	B	B	48,XY,+15,+19	Abnormal
		19	4	B	B	45,XX,-22	Abnormal
98	40	1	5	B	B	46,XY	Normal
		2	4	A	B	45,XX,-19	Abnormal
99	40	1	4	A	B	47,XX,+15	Abnormal
		2	4	A	B	47,XX,+15	Abnormal
		3	4	B	B	43,XY,-4,-5,-22	Abnormal
		7	4	B	B	45,XX,-3pter-3p21.2,+3q21.1-3qter,-22	Abnormal
		C2_6	3	B	B	47,XY,+16	Abnormal
		C2_9	3	B	B	47,XX,+16	Abnormal
		C2_11	5	A	A	47,XX,+15	Abnormal
100	40	1	4	A	A	47,XY,+11	Abnormal
		2	4	A	A	46,XY	Normal
		3	4	A	A	46,XX	Normal
		4	4	A	A	45,XY,-18	Abnormal
		5	5	A	A	45,X0	Abnormal
		6	4	B	C	46,X0,+12	Abnormal
101	40	2	1			46,XY	Normal
		3	1			45,XX,-22	Abnormal
		C2_1	5	C	C	46,XX	Normal
		C2_2	2	B	C	45,XXY,-5,-12	Abnormal
		C2_3	1	C	C	46,XX,+6q11-6q23.3	Abnormal
		C2_4	1	D	C	46,XY	Normal

		C2_5	1	D	C	47,XY,+4	Abnormal
		C2_6	1	D	D	46,XX	Normal
		C2_7	0			45,Y0,-X	Abnormal
102	40	1	2			46,XX	Normal
		4	2			47,XY,+11	Abnormal
		5	2			44,XX,-13,-16	Abnormal
103	40	1	5	B-	C	No result	Undiagnosed
		4	4	C	C	48,XX,+6,+13	Abnormal
		14	5	C	C	47,XX,+19p13.3_p12	Abnormal
104	41	7	3	B	B	48,XY,+16,+22	Abnormal
		8	3	B	B	46,XX	Normal
		9	4	B	B	47,XY,+19	Abnormal
105	41	9	2	B	B	45,XY,-22	Abnormal
106	41	3	4	B	A	No result	Undiagnosed
		4	4	B	A	46,XX	Normal
		5	4	B	B	45,XX,-12	Abnormal
		11	4	A	A	46,XY	Normal
		12	4	A	A	No result	Undiagnosed
107	41	1	4	A	B	47,XY,+22	Abnormal
		2	3	A	B	46,XY,+2,-4	Abnormal
		3	3	A	B	46,XX	Normal
		4	3	A	B	48,XY,+1,+15	Abnormal
		5	5	A	B	No result	Undiagnosed
108	41	C2_1	4	B	B	45,XX,-14	Abnormal
		C2_2	3	B	A	47,XY,+15	Abnormal
		C2_3	3	B	B	45,XX,-19	Abnormal
109	41	1	4	B	B	46,X0,+18	Abnormal
		2	4	A	A	47,XX,+5,+15	Abnormal
		3	4	B	B	46,XY,+17,-22	Abnormal
		4	4	A	A	46,XY	Normal
		5	2/3			46,XY,+11pter-11p14,-11p13-11qter,+17,-22	Abnormal
		6	3	B	B	45,XY,-16	Abnormal
		7	2/3			47,XY,+15,+22	Abnormal
		8	3	A	B	46,XX	Normal

		9	5	A	B	46,XX	Normal
		10	5	A	B	45,XY,+11,-16,-22	Abnormal
		11	6	B	B	46,XX	Normal
		12	2/3			45,XY,-3	Abnormal
110	41	1	4	A	B	46,XY	Normal
		2	2/3			47,XY,+2	Abnormal
		3	2/3			44,XX,-8,-21	Abnormal
		4	2/3			47,XX,-11,+19,+22	Abnormal
		5	4	B	B	SG failure/ No result	Undiagnosed
		6	4	C	B	48,XX,+15,+18	Abnormal
		7	4	A	A	46,XX	Normal
		8	2/3			46,XY	Normal
111	41	2	3	B	B	No result	Undiagnosed
		5	2/3			46,XX,-16,+22	Abnormal
		6	2/3			46,XY,+1q31-q44	Abnormal
		7	4	A	B	47,XY,+19	Abnormal
		10	3	B	B	46,XY,+10,+19,-20,-21	Abnormal
		11	4	B	B	46,XX	Normal
		12	3	A	A	45,XX,-4	Abnormal
		13	3	B	B	47,XY,+22	Abnormal
112	41	6	4	B	B	46,X0,+22	Abnormal
		8	3	B	B	46,XY	Normal
		10	3	B	B	48,Y,+1,+12,+17,-X	Abnormal
		11	3	A	A	47,XX,+21	Abnormal
113	41	1	4	A	A	46,XX	Normal
		2	3	A	A	45,XY,-15	Abnormal
		3	3	A	A	47,XY,+4,-18,+22	Abnormal
		4	4	B	B	46,XX	Normal
		5	3	B	A	45,XY,-15	Abnormal
		6	3	B	A	44,XY,-16,-18	Abnormal
		7	3	B	A	45,XX,+2,+9pter-p11,-17,-22	Abnormal
		8	3	B	B	46,XY	Normal
		9	2/3			45,XY,-10	Abnormal
		18	4	B	A	46,XX	Normal

		19	3	B	B	47,XX,+3,-9,+16	Abnormal
114	41	1	4	A	A	SG failure/ No result	Undiagnosed
		2	2/3			45,XX,-18	Abnormal
		3	4	A	B	47,XYY	Abnormal
		4	4	B	C	46,XY	Normal
		5	4	A	A	46,XY	Normal
115	41	2	2/3			46,XX	Normal
		4	5	A	B	48,XY,+16,+21	Abnormal
		5	2/3			46,XY,-8,+22	Abnormal
		7	4	B	B	45,XO,-4,+22	Abnormal
		9	4	B	B	46,XY	Normal
116	41	1	3	B	B	44,XY,+2,-7,-13,-21	Abnormal
		2	2/3			46,XY	Normal
		3	3	A	A	46,XY	Normal
		5	3	A	B	47,XY,+16	Abnormal
		7	4	A	A	47,XY,+22	Abnormal
		12	3	B	B	46,XY,-3p26-3p11	Abnormal
		13	4	A	A	46,XX,-14,+16	Abnormal
		14	3	B	B	44,XY,-16,-21	Abnormal
		15	3	A	B	46,XY	Normal
117	41	6	4	B	B	45,XY,-21	Abnormal
		7	4	B	A	46,XY	Normal
118	41	1	4	A	B	46,XX	Normal
		12	2/3			44,XX,-11,-18	Abnormal
		14	2/3			45,XX,-14	Abnormal
		15	4	B	B	46,XX	Normal
		19	3	A	B	44,XX,-9,-11	Abnormal
		20	4	B	B	46,XX	Normal
		21	4	B	B	47,XY,-7q11.2-q36,+18	Abnormal
119	41	3	3	B	B	45,XX,-15	Abnormal
		6	4	B	A	47,XX,+22	Abnormal
		8	3	B	B	46,XX	Normal
		11	3	B	B	46,XY	Normal
		18	1			46,XX	Normal

120	41	1	4	B	B	47,XX,+1	Abnormal
		2	3	A	B	44,XY,-14,-16,-21,+22	Abnormal
		4	4	B	B	47,XXY,-14	Abnormal
		21	3	B	B	45,XX,-16	Abnormal
		22	3	B	B	47,XX,+21	Abnormal
		23	4	A	B	46,XX,+15,-22	Abnormal
		24	3	A	B	45,XY,-16	Abnormal
121	41	4	2/3			47,XXY	Abnormal
		8	3	A	A	46,XY	Normal
		10	2/3			45,XX,+8,-18,-22	Abnormal
		11	2/3			46,XY	Normal
122	41	4	2/3			46,XY	Normal
		6	4	B	A	47,XX,+20	Abnormal
		8	5	B	B	46,XY,-5q11.1-qter,+8pter-8p11.1,-8q11-8qter,-11,+16	Abnormal
		9	5	B	A	46,XX	Normal
		10	4	A	A	45,XY,-21	Abnormal
123	41	3	3/4	B-	C-	46,XY,-19,+22	Abnormal
		7	4	B-	C-	47,XX,+22	Abnormal
124	41	4	3	B	B	No result	Undiagnosed
		6	3	B	B	46,XY	Normal
		8	3	B	B	45,XX,-10	Abnormal
125	41	1	3	B	B	47,XY,+10	Abnormal
		3	3	B	B	No result	Undiagnosed
		4	3	B	B	46,XY	Normal
126	41	1	2			46,XY	Normal
		2	3	A	B	47,XY,-2p,+15	Abnormal
		3	2			45,XX,-9	Abnormal
		4	3	B	B	47,XY,-4p16.3_q33,+4q33_q35.2,+8,-13	Abnormal
127	41	6	4	B	A	45,XX,-14	Abnormal
128	42	4	4	A	B	47,XY,+22	Abnormal
		8	3	A	B	46,XX	Normal
129	42	2	4	A	A	47,XY,+15	Abnormal
		8	4	A	A	47,XX,+22	Abnormal
		12	3	A	B	46,XX	Normal

		13	3	A	A	46,XY,-16q11.1-q24	Abnormal
		C2_3	4	A	B	46,XX,+4,-20	Abnormal
		C2_5	3			45,XY,-15	Abnormal
		C2_7	4	A	A	46,XY	Normal
		C2_10	4	A	A	46,XX	Normal
		C2_15	4	A	A	46,XX	Normal
		C2_20	3	B	B	47,XX,+17	Abnormal
		C3_1	4	A	B	45,XY,-16	Abnormal
		C3_2	4	B	C	46,XY,-18,+22	Abnormal
		C3_4	6	A	B	46,XX	Normal
130	42	2	3	A	B	46,XX,-5,+20	Abnormal
		5	3	B	B	46,XX,-1,+16	Abnormal
131	42	3	3	A	B	46,XY	Normal
		9	3	A	A	46,XX	Normal
		16	3			43,XX,-7,-15,-20	Abnormal
132	42	1	3	A	A	45,XO	Abnormal
		2	3	A	A	46,XX	Normal
		3	3	A	B	No result	Undiagnosed
		4	6	B	B	46,XY	Normal
		5	4	B	B	47,XX,+8	Abnormal
		6	4	A	B	47,XX,+19	Abnormal
		9	5	A	B	47,XY,+22	Abnormal
133	42	1	3	B	B	46,XY	Normal
		6	4	A	B	45,XX,-2,-7,+11	Abnormal
		10	4	A	B	46,XY	Normal
		11	4	A	B	46,XY	Normal
		12	3			47,XXY	Abnormal
		13	4	B	C	45,XO	Abnormal
		14	4	A	C	48,XXYY	Abnormal
134	42	1	5	B	B	47,XY,+17	Abnormal
135	42	1	2/3			46,XY	Normal
		2	3	A	B	No result	Undiagnosed
		3	3	A	B	47,X0,+4,+17	Abnormal
		4	2/3			44,XX,-14,-15	Abnormal

		5	4	B	A	46,XX	Normal
		6	2/3			46,XY	Normal
		7	4	A	B	47,XX,+6	Abnormal
		8	3	B	B	44,XX,-9,-15	Abnormal
		9	3	B	B	46,XX	Normal
		10	3	B	B	No result	Undiagnosed
		11	4	B	B	46,XY,-14,+21	Abnormal
		12	4	B	B	46,XY,+16,-20	Abnormal
		13	3	A	B	No result	Undiagnosed
		14	4	B	A	No result	Undiagnosed
		15	2/3			47,XY,-14,+16,+20	Abnormal
		16	4	A	B	No result	Undiagnosed
136	42	1	3	B	A	No result	Undiagnosed
		2	4	A	B	46,XY	Normal
		3	4	B	A	46,XY	Normal
		4	2/3			46,XY	Normal
137	42	1	3	B	B	46,XY,+15,-18	Abnormal
		2	3	A	B	47,XY,+17	Abnormal
		3	2/3			45,XY,-6	Abnormal
		4	4	B	B	46,XY,+12,-15	Abnormal
138	42	1	4	A	A	46,XY	Normal
		2	3	B	B	46,XY	Normal
		3	4	A	B	44,X0,-13	Abnormal
139	42	1	3	B	C	46,XY	Normal
		2	2/3			44,XY,-18,-21	Abnormal
		3	3	C	C	47,XX,+6	Abnormal
		4	3	B-	C-	47,XX,+12	Abnormal
		5	2			No result	Undiagnosed
		6	3	B-	C-	No result	Undiagnosed
		7	2/3			45,XX,-18	Abnormal
		8	2			48,XX,+8,+15	Abnormal
140	42	1	4	A	B	46,XX,-11,+13	Abnormal
		2	4	B	B	46,XX	Normal
		3	4	C	B	45,XY,-5,-6,-7,+8,+10,+11,+13,-14,-15,-16,-17,+18,-	Abnormal

						20,+21,+22	
		4	3	C	C	No result	Undiagnosed
141	42	1	2	C	C	46,XX,+8,-18	Abnormal
142	42	1	3	C+	C	47,X0,+9+20	Abnormal
143	43	1	3	B	C	47,XX,+16	Abnormal
		2	3	B	B	46,XXY,-22	Abnormal
		3	2	B	B	45,XY,-14	Abnormal
144	43	1	3	B	B	45,XY,-4,+16,+22	Abnormal
		2	3	A	B	No result	Undiagnosed
		3	4	A	A	46,XY	Normal
145	43	1	3	B	B	46,XY	Normal
146	43	1	5	B	C	46,XY,+15,-22	Abnormal
147	43	1	3	B	B	46,XX	Normal
		2	2/3			47,XY,+15	Abnormal
		3	4	B	B	46,XY	Normal
		4	2/3			43,XX,-9,-11,+13,-16,-18	Abnormal
148	43	1	4	B	B	47,XY,+4,+5,+9p24-q22.1,-11,+16,-21	Abnormal
		2	2/3			47,XX,+1	Abnormal
		3	3	A	B	46,XY,-11,+21	Abnormal
		4	5	B	B	47,XY,+19	Abnormal
		5	3	B	B	45,XX,-1p36.3-p11,+1q11-q44,-12,-13,+14	Abnormal
		6	4	B	B	46,XO,-11,+19,+22	Abnormal
		7	4	A	B	45,XX,-8	Abnormal
		8	2/3			45,XY,-1,-16,+17	Abnormal
149	43	1	4	A	A	46,XX	Normal
		2	2/3			48,XX,+4,+6	Abnormal
		3	4	B	B	45,XX,+4q13.3-qter,-7,-15,+18	Abnormal
150	43	1	3	b	b	44,XY,-2,-15	Abnormal
		2	2/3			46,X0,+2,+7,+8,-9,-15,+16,+17	Abnormal
		3	3/4			45,XY,-18	Abnormal
		4	4	A	A	47,XY,+12	Abnormal
		5	4	B	C	45,XY,+5,-11,-15,-19,+22	Abnormal
		6	3	B	C	46,XX,+3,-12,-13,-20,+21,+22	Abnormal
151	43	1	2/3			No result	Undiagnosed

		2	1			46,XX	Normal
		3	4	B	A	No result	Undiagnosed
		4	3	A	B	45,XY,-18	Abnormal
		5	1			No result	Undiagnosed
152	43	1	5	B	A	49,XX,+16,+19,+20	Abnormal
		2	4	B	A	45,XX,-21	Abnormal
		3	4	A	A	45,XX,-22	Abnormal
		4	4	B	B	45,XY,+15,-18,-19	Abnormal
		5	3	A	A	46,XY,+14,-16,+18,-22	Abnormal
		6	4	B	B	47,XY,-10,+15,+22	Abnormal
153	43	1	3	A	B	46,XY	Normal
		2	4	B	B	45,XX,-20	Abnormal
154	43	1	3	A	B	47,XY,+9,+19	Abnormal
		2	2/3			44,XY,-11,-21	Abnormal
		3	3	C	B	45,XX,-15,+16,-21	Abnormal
155	44	1	4	A	B	No result	Undiagnosed
		2	3	A	B	44,XY,-2,-3,+10,-21	Abnormal
		3	3			48,XYY,-14,+16,+17	Abnormal
		4	3	B	A	45,XO,+16,-21	Abnormal
156	44	1	5	B	B	47,XY,+15,+17,-21	Abnormal
		2	2/3			46,XY	Normal
		3	3	A	B	48,XY,+11,+16	Abnormal
157	44	1	5	A	B	45,XY,-13	Abnormal
158	44	1	4	B	A	No result	Undiagnosed
		2	2/3			41,XX,-2,-4,+7,-8,-9,+10,-11,-13,+15,-18	Abnormal
		3	4	B	B	46,XX	Normal
		4	2/3			47,XX,+15	Abnormal
		5	2/3			46,XY,-13,-15,+17,+22	Abnormal
		6	3	B	A	46,XY	Normal
		7	4	A	A	45,XY,-18,-20,+21	Abnormal
		8	4	B	A	46,XX	Normal
		9	4	A	B	45,XX,-13	Abnormal
159	44	1	3	A	B	46,XX,+4,-7,+12,-22	Abnormal
160	45	1	3	B	C	46,XY,+16,-22	Abnormal

		2	1			47,XY,+13	Abnormal
161	46	1	3	A	A	46,XX,-9,+17	Abnormal
		2	3	B	A	47,XX,+15	Abnormal
		3	3			45,XX,-3,-7,-10	Abnormal
		4	4	A	A	48,XX,+12,+17	Abnormal
		5	4	A	A	45,XO	Abnormal
		6	4	B	B	46,XX	Normal
		7	4	A	B	No result	Undiagnosed
		8	4	B	B	No result	Undiagnosed
		9	4	B	B	45,XX,-19	Abnormal
162	47	1	3			47,XX,+22	Abnormal
		2	3	B	C	46,XX,+15,-22	Abnormal
		3	3	A	B	45,XX,-7q33-qter,-10	Abnormal

Appendix C

Table C-1: Results of PGD case for translocation and inversion carriers

Case	Karyotype (and the size of rearranged fragments)	Stage tested	Embryo number	Embryo karyotype	Characterization
1	46,XY,t(17;19)(q25.3;q13.1) (chromosome 17 fragment 6 Mbp; chromosome 19 fragment 36Mbp)	Bla	1	43,X0,-4,-8,-17p13.1-17q25.3,+19q13.12-q13.43	Abnormal
			2	46,XX	Normal/Balanced
			3	46,XX,-1p36.33-1p32.2,-17q25.1-17q25.3,-19q13.12-19q13.43	Abnormal
			4	46,XX,-19q13.11-19q13.43	Abnormal
			5	45,XX,-20	Abnormal
			6	48,XX,+11,+22	Abnormal
			7	45,XY,-17,+19p13.3-19q13.12	Abnormal
2	46,XY,t(17;19)(q25.3;q13.1) (chromosome 17 fragment 6 Mbp; chromosome 19 fragment 36Mbp)	Bla	1	46,XY,-17q25.3-q25.1, 13.1-q13-43	Abnormal
			2	46,XX,-19q13.1-q13.43	Abnormal
			3	45,XX,-10,+17p13.3-p11.2,-17p11.2-q25.1,-19q13.12-q13.43	Abnormal
			4	46,XX,-19p13.3-q13.12	Abnormal
			5	46,XY,-19q12-q13.43	Abnormal
			6	46,XX	Normal/Balanced
			7	46,XX,-19q13.12-q13.43	Abnormal
			8	46,XX	Normal/Balanced
			9	47,XY,+3,+4p16.3-p14,-4p14-q32.2,+13,-22	Abnormal
			10	45,XY,-5	Abnormal
			11	46,XX,+19q13.1-13.43	Abnormal
3	46,XY,t(16;17)(q11;p13.3) (chromosome 16 fragment 51 Mbp; chromosome 17 fragment 3.6Mbp)	Bla	1	41,XX,-1,-4,-6,-7,-9,+11,+16q12.1-q24.3,+17,-18,-19,+20,+21,-22	Abnormal
			2	45,XY,-1p36.33-1p36.13,+4q22.3-4q35.2,-10	Abnormal
			3	46,XY,-10p15.3-q22.3,+10q22.3-q26.3,-16q12.1-q24.33	Abnormal
			4	44,XY,-4,-8	Abnormal
			5	46,XY	Abnormal
			6	no result	No diagnosis
4	45,XY,(13;14)(q10;q10)	TE	1	45,XY,-13	Abnormal
			2	47,XY,+14	Abnormal
			3	46,XY	Normal/Balanced
			4	47,XX,+11	Abnormal
			5	45,XX,-13	Abnormal
			6	46,XY	Normal/Balanced
			7	46,XX	Normal/Balanced

			8	45,XY,-7,+8,-13,+16,-17	Abnormal
			9	47,XX,+20	Abnormal
			10	No result	No diagnosis
			11	45,XX,-22	Abnormal
			12	48,XX,+3,+5	Abnormal
			13	47,XY,+14	Abnormal
			14	46,XX	Normal/Balanced
5	45,XY,(13;14)(q10;q10)	TE	1	46,XX	Normal/Balanced
			2	47,XY,+15	Abnormal
			3	46,XX	Normal/Balanced
			4	46,XY	Normal/Balanced
			5	45,XY,-15	Abnormal
			6	45,XX,-14	Abnormal
			7	46,XX	Normal/Balanced
			8	46,XY	Normal/Balanced
			9	46,XX	Normal/Balanced
6	46,XY,t(1;2)(q23.1;q35) [chromosome 1 fragment: 87 Mbp; chromosome 2 fragment: 20 Mbp]	TE	1	46,XY,-1p36.33-p34,+1q23.3-q44,-2q36.2-q37.3	Abnormal
			2	46,XY,-1q23.3-q44,+2q36.2-q37.3	Abnormal
			3	46,XY,-1q23.3-q44,+2q36.2-q37.3	Abnormal
7	46,XY,t(1;2)(q23.1;q35) [chromosome 1 fragment: 87 Mbp; chromosome 2 fragment: 20 Mbp]	Bla	1	49,XY,+9,+11,+17	Abnormal
			2	46,XX,-1q23.3-1q44,+2q36.1-2q37.3,-13,+21	Abnormal
			3	45,XX,-1q23.3-1q44,-2p25.3-2q35,+4q28.3-4q35.2,-15	Abnormal
			4	46,XX,+1q23.3-1q44,-2q36.2-2q37.3	Abnormal
			5	43,XY,+1q23.3-1q44,-2q36.2-2q37.3,-4,+5,-6,+7q11.23-7q36.3,-8,-10	Abnormal
8	45,XY,der(14;21)(q10;q10)	TE	1	46,XY	Normal/Balanced
			2	42,XY,-7,-16,-17,-18	Abnormal
9	45,XY,der(13;14)(q10;q10)	Bla	1	50,XYY,+8, +15,+16,+19,-21,+Y	Abnormal
			2	45,X0	Abnormal
			3	46,XX	Normal/Balanced
			4	47,XX,+1p13.2-1q44,-3p26.3-3p26.1,+3p26.1-3p12.2,+11	Abnormal
10	46,XY,t(1;3)(q31;p13) [chromosome 1 fragment: 58 Mbp; chromosome 3 fragment: 89 Mbp]	TE	1	46,XY	Normal/Balanced
			2	46,XY,+1q31-q44,-3p26.3-p13	Abnormal
			3	46,XX,-1q31-q44,+3p26.3-p13	Abnormal
			4	50,YY,-7,+9,-	Abnormal

				10,+11,+13,+14,+16,+18,+20	
			5	46,XX	Normal/Balanced
			6	46,XX	Normal/Balanced
			7	46,XY,+5,-7	Abnormal
11	45,XY,der(14;21)(q10;q10)	Bla	1	47,XX,-4,+13,+14,-15,+16	Abnormal
			2	46,XX	Normal/Balanced
12	46, XY, t(5;15)(q13.1;q21.2)/inv(3)(q25.1;q26.2) [chromosome 5 fragment: 112 Mbp; chromosome 15 fragments: 57 Mbp]; [paracentric inversion on chromosome 3: 50 Mbp]	Bla	1	47,XX,-5q13.1-q26.3,+15	Abnormal
			2	44, XX,+3,-5q13.1-q26.3,+6,-9,-10,+12,-14,+15,-20,-21,-22	Abnormal
			3	46,XY,-10q11-q26.3	Abnormal
			4	50,XY,-1,-2,+3,-4,+5,-6,-7,+8,+9,-10,+11,+13,+14,+15,+18,+19,-21,+22	Abnormal
			5	47, XY,+22	Abnormal
			6	46,XX,+5q13.1-q26.3,+15,-16	Abnormal
			7	46,XY,+5q13.1-q26.3,-15	Abnormal
			8	47,XY,-3p26.3-p11.1,+5,+6,-7,-8,-11,+13,+16,+18,-20	Abnormal
13	46,XY,t(11;22)(q23;q11.2) [chromosome 11 fragment: 19 Mbp; chromosome 22 fragment: 28 Mbp]	TE	1	47,XX,-11q23-qter,+22q11.2-22qter	Abnormal
			2	45,XX,+11q23-qter,-22q11.2-22qter	Abnormal
			3	46,XX	Normal/Balanced
			4	45,XY,+11q23-qter,-22q11.2-22qter	Abnormal
			5	46,XX	Normal/Balanced
			6	46,XX,-11q23-qter,-18,+22q11.2-22qter	Abnormal
			7	45,XX,+11pter-11q23, -22q11.1-q11.21	Abnormal
			8	45,XX,-18	Abnormal
			9	46,XY	Normal/Balanced
14	45,XY,der(13;15)(q10;q10)	TE	1	46,XY,-13,+22	Abnormal
			2	45,XY,-4	Abnormal
			3	47,XYY	Abnormal
			4	No result	No diagnosis
			5	45,XY,-13	Abnormal
			6	47,XX,+22	Abnormal
			7	45,XX,-13	Abnormal
			8	No result	No diagnosis
			9	No result	No diagnosis
			10	No result	No diagnosis
			11	47,XY,+16	Abnormal
			12	46,XY,-10,+15	Abnormal

			13	45,XY,-13	Abnormal
			14	45,XX,-13	Abnormal
			15	47,XX,+1	Abnormal
case 15	46,XY,t(13;15)(q21.3;q11.2)	Bla	1	no result	No diagnosis
			2	47,XY,+6	Abnormal
			3	45,X0,-13q21.3-q34,+15,-16	Abnormal
			4	46,XY,-13q21.3-q34	Abnormal
			5	no result	No diagnosis
case 16	45,XX,der(13;15)(q10;q10)	Bla	1	40,XY,-2,-9,-13,-16,-17,-20	Abnormal
			2	46,XX	Normal/Balanced
			3	No result	No diagnosis
			4	45,XX,-7,+15,-21	Abnormal
			5	45,XX,-15	Abnormal
			6	48,XX,+6,+13,+19p	Abnormal
			7	54,XY,+1,+4,+6,-8p23.11-q11.23,-9,+10,+11,+12,+13,-14,+15,+16,+17,+18,-20	Abnormal
			8	46,XY	Normal/Balanced
case 17	46,XX,t(17;18)(p11.2;p11.2) [chromosome 17 fragment: 22 Mbp; chromosome 18 fragment: 15.4 Mbp]	Bla	1	45,XX,+15,-17p13.3-p11.2	Abnormal
			2	46,XX,+17p11.2-q25.3,-18p11.2-q23	Abnormal
			3	46,XY	Normal/Balanced
case 18	46,XX,t(8;9)(p12;q31) [chromosome 8 fragment: 39 Mbp; chromosome 9 fragment: 38 Mbp]	TE	1	48,XX,+13,+19	Abnormal
			2	No result	No diagnosis
			3	46,XX	Normal/Balanced
			4	46,XY,+2q34-q37.3	Abnormal
			6	46,XX	Normal/Balanced
			7	No result	No diagnosis
			8	46,XY,-9q33.1-q34.3	Abnormal
case 19	46, XY, t(1;8)(q25.3;p11.2) [chromosome 1 fragment: 68 Mbp; chromosome 8 fragment: 40 Mbp]	Bla	1	No result	No diagnosis
			2	46, XX	Normal/Balanced
			3	45, XX,-1q25-q44,-5q34-q35.3,-8p11.2-q24.3,-10	Abnormal
			4	No result	No diagnosis
case 20	46, XX, t(10;16)(q11.2;p11.2) [chromosome 10 fragment: 89 Mbp; chromosome 16 fragment: 24 Mbp]	Bla	1	46,XX	Normal/Balanced
			2	46,XY	Normal/Balanced
			3	46,XY	Normal/Balanced
			4	48,XY,-1p36.33-p12,+1q21-q44,-10q11.2-q24.3,+14,+16p13.3-p11.2,+17	Abnormal

			5	46,XY,+8,+10q11.2-q24.3,-16p13.3-p11.2,-19	Abnormal
case 21	46,XX,t(1;2)(q24;p21) [chromosome 1 fragment: 83 Mbp; chromosome 2 fragment: 48 Mbp]	Bla	1	42,X0,-4,-6,+10,+11,-17,-19p12-19q13.43,-20,-22	Abnormal
			2	44,X0,+5,-6,+11q21.1-11q25,-15,-16,+18,-19,+22	Abnormal
			3	52,XX,+1p36.33-1q42.13,+2p25.3-2p21,+4,+7,+9,+10,+13,+21	Abnormal
case 22	46,XX,t(1;2)(q24;p21) [chromosome 1 fragment: 83 Mbp; chromosome 2 fragment: 48 Mbp]	TE	11	46,XX	Normal/Balanced
			13	46,XX	Normal/Balanced
case 23	45,XY,der(13;14)(q10;q10)	Bla	1	46,XY	Normal/Balanced
			2	52,Y0,-1,-2,+3,+4,-5,+7,+8,+9,+11,+12,+15,+16,-17,+19,+20,+21,-22,-X	Abnormal
			3	45,X0,-13,+16	Abnormal
			4	47,XX,+21	Abnormal
			5	48,XY,+13,+14	Abnormal
			6	45,XX,-6	Abnormal
			7	45,X0	Abnormal
case 24	46,XX,t(1;5)(p36.1;q33) [chromosome 1 fragment: 28 Mbp; chromosome 5 fragment: 33.6 Mbp]	TE	1	46,XY,+1p36.33-p36.1,-5q33-q35.3	Abnormal
			2	46,XX,+1p36.33-p36.1,-5q33-q35.3	Abnormal
			3	46,XX,-1p36.33-p36.1,+5q33-q35.3	Abnormal
			4	46,XX,+1p36.33-p36.1,-5q33-q35.3	Abnormal
			5	46,Y0,-1p36.13-q44,+5p15.33-q33.3,-X	Abnormal
case 25	46,XX,t(1;5)(p36.1;q33) [chromosome 1 fragment: 28 Mbp; chromosome 5 fragment: 33.6 Mbp]	TE	1	46,XX,+1p36.33-p36.13,-5q33.2-q35.3	Abnormal
			2	46,XY	Normal/Balanced
			3	45,XX, -1p36.33-p36.13,-14	Abnormal
			4	46,XY,+1p36.33-p36.13,-3q24-q29,-5q33-q35.3	Abnormal
case 26	46,XY,inv(8)(p21q24.1) [Pericentric inversion, fragment size: 108 Mbp; Short arm fragment (8pter–8p21): 30 Mbp; Long arm fragment (8q24.1–8qter): 29 Mbp]	TE	1	45,XY-20	Abnormal
			2	46,XX	Normal/Balanced
			3	46,XY	Normal/Balanced
case 27	46,XY,t(10;17)(q21.2;p11.2) [chromosome 10 fragment: 74 Mbp; chromosome 17 fragment: 22 Mbp	Bla	1	47,XY,-10q22.3-10q26.3,+17	Abnormal
			2	48,XX,+16,+21	Abnormal
			3	47,XX,+2,+10q22,3-10q26.3,-16p13.2-16p12.1	Abnormal
			4	No result	No diagnosis
case 28	45,XY,der(13;14)(q10;q10)	Bla	1	47,XY,+15	Abnormal

			2	41,00,-2,+3,-5,+10,-11,-12,+13,+14,+19,-X,-Y	Abnormal
case 29	46,XY,t(7;18)(p15.3;q12.2) [chromosome 7 fragment: 25 Mbp; chromosome 18 fragment: 45 Mbp	Bla	1	46,XX,-7p21.1-q36.3,+18p11.32-q12.3	Abnormal
			2	45,XX,-22	Abnormal
			3	46,XY	Normal/Balanced
			4	46,XX,-4p16.3-p12,-7p22.3-p21.3,-13q33.3-34,+18q21.2-q23	Abnormal
case 30	45,XY,der(13;14)(q10;q10)	Bla	1	47,XX,-10q26.2-q26.3,-20q13.2-q13.33,+22	Abnormal
			2	45,XX,-10	Abnormal
			3	46,XY	Normal/Balanced
			4	53,YY,+1,-4,+5,+9,-10,+12,+14,+16,+17,+20,+21,+22,-X	Abnormal
			5	46,XX,-7	Abnormal
case 31	45,XX,der(13;14)(q10;q10)	Bla	1	47,XY,+7	Abnormal
			2	43,XX,-1, -2, -3, +4, +5, -6, -7, -11, -12, +13, +18	Abnormal
			3	55,XXX,-3, +9, +10, +11, +12, +13, +18, +19, +20, +22	Abnormal
			4	47,XY, +13, +14, -19	Abnormal
			5	47,XXY,+4, -14	Abnormal
			6	44, XY,-4,-17	Abnormal
			7	undiagnosed	No diagnosis
			8	50, XX,-1,+2,-3,-5,+6,+7,-8,+9,+10,+11,+12,+13,+14,-15,-16,+18,+20,-22	Abnormal
			9	48,XXY,+3	Abnormal
case 32	45, XX, der(13;21)(q10;q10)	Bla	1	46,XX	Normal/Balanced
			2	48,XX,+13,+21	Abnormal
			3	48,XX,-2,+3,-4,+6,-12,+15,+21,+22	Abnormal
			4	45,XX,+1,-8,-9,+11q13.2-q25,-12,+13,+14,-16,+17,+18,-21,-22	Abnormal
			5	47,XX,+6	Abnormal
			6	43,X0,-4,-8,	Abnormal
case 33	45, XX, der(13;21)(q10;q10)	Bla	1	46,XX,+2q11.2-q37.3,+16,-21	Abnormal

			2	45,XX,-19	Abnormal
			3	46,XX,+16,-21	Abnormal
			4	48,XY,+21,+22	Abnormal
			5	46,XY	Normal/Balanced
case 34	46, XY, t(1;3)(q31;p13) [chromosome 1 fragment: 58 Mbp; chromosome 3 fragment: 89 Mbp]	Bla	1	41,X0,-1,+2,+3,-5,+8,-13,-14,-15,-16,+17,-18,+20q13.1-q13.33,-22	Abnormal
			2	46,XX,-1q31-q44,-3p13-q29	Abnormal
			3	45,XX,-2	Abnormal
			4	46,XX,-3p26.3-q10,+3q10-q29	Abnormal
			5	47,YY,+1,-3,-6,+12,+13,-14,+15,+16p11.2q24.2,+17,+18,-22	Abnormal
Case 34 (continue)			6	No result	No diagnosis
			7	No result	No diagnosis
			8	48,XX,-3p26.3-p13,+13,-16,+19,+22	Abnormal
			9	46,XY,+1q31-q44,+3p13-q26	Abnormal
			10	46,XY,+1q31-q44,+3p13-q26	Abnormal
			11	47,XX,-1q31-q44,+3p13-q29,+6	Abnormal
case 35	45,XY,der(13;14)(q10;q10)	Bla	1	45,XX,-15	Abnormal
			2	47,XX,+14	Abnormal
			3	43,XX,-1,-2,-4	Abnormal
			4	40,XY,+1,-2,-3,+4,+5,-6,+7,-8,+9,-10,-11,-12,-13,-14,+15,-16,-17,+19,-20,-21,-22	Abnormal
			5	49,XX,+3,+6,+7,-8,+18	Abnormal
			6	45,XY,-16	Abnormal
case 36	45,XY,der(14;21)(q10;q10)	Bla	1	46,XX,-12q23.2-q24.33	Abnormal
			3	46,XX,-5,+14	Abnormal
			5	No result	No diagnosis
case 37	45,XY,der(14;21)(q10;q10)	Bla	1	47,XY,-15,+16,+22	Abnormal
			2	46,XX	Normal/Balanced
			3	50,XX,+1,-3,+4,+6,+8,-11,-12,+13,+14,-17,+18,+20,+21,-22	Abnormal
			4	48,XX,+9,+17	Abnormal
			5	46,XXY	Abnormal
			6	44,XY,-3,-21	Abnormal
			7	44,X0,-2q22.1-q37.3,+10q21.3-26.3,-18,-X	Abnormal
			8	47,XX,+13,-15,+17	Abnormal
case 38	46,XY,t(17;21)(q21;q22) [chromosome 17 fragment: 44 Mbp; chromosome 3 frag-	Bla	1	47,XY,+16	Abnormal

	ment: 16.5 Mbp]		2	47,XY,-7,+14,+22	Abnormal
case 39	46,XY,t(1;15)(q21p11.2) [chromosome 1 fragment: 89 Mbp; chromosome 15 fragment: 57 Mbp]	Bla	1	47,XXY	Abnormal
			2	48,XX,+1,+12,-16	Abnormal
			3	46,XY,1pter-1p11, +1q11-1qter,-6q11-6qter	Abnormal
			4	43,XY,-2,+5pter-5p11,-13.-17	Abnormal
			5	46,XX	Normal/Balanced
Case 39 (con-tinue)			6	47,XY,+11	Abnormal
			7	47,XX,+20	Abnormal
			8	No result	No diagnosis
case 40	46, XX, t(7;10)(p11.2;p11.23) [chromo-some 7 fragment: 57 Mbp; chromosome 10 fragment: 31 Mbp]	Bla	1	46,XX	Normal/Balanced
			2	46,Y0,+17	Abnormal
			3	45,XY,-12	Abnormal
case 41	46, XY, t(5;7)(q23.2;p14) [chromosome 5 fragment: 51 Mbp; chromosome 7 frag-ment: 38 Mbp]	TE	1	45,XY,-4,+5q23.2-q35.3,-7p22.3-p14	Abnormal
			2	46,XY	Normal/Balanced
			3	47,XXY,+5q23.2-q35.3,-7p22.3-p14,-16,+19	Abnormal
case 42	45,XX,der(13;14)(q10;q10)	TE	1	47,XY,+21	Abnormal
			2	48,Y0,+1,-2,+3p26.3-p14.1,-8,+12,+13,+14,+16,-18,+20,+21	Abnormal
			3	48,XX,+9,+15,-16,+22	Abnormal
			4	47,XY,+13,-14,+20	Abnormal
			5	47,XY,+16	Abnormal
case 43	45,XX,(13;14)(q10;q10)	PB (1st and 2nd)	1	24,X,+14	Abnormal
			2	23,X,+11,-16	Abnormal
			3	26,X,+6,-12,+13,-15,+18,+21,+22	Abnormal
			4	24,X,-13,+21,+22	Abnormal
			5	21,X,-14,+16,-18	Abnormal
			6	No result	No diagnosis
			7	23,X	Normal/Balanced
			8	23,X,+14,+15,-16,-21	Abnormal
			9	22,X,-15	Abnormal
			10	23,X,-13,+15	Abnormal
case 44	45,XX,(13;14)(q10;q10)	PB	1	22,X,-9cht,-14,+15 at metaphase II	Abnormal
			2	24,X,+11	Abnormal
			3	22,X,-1,+8,-19	Abnormal
			4	25,X,+13,+14	Abnormal

			5	21,X,-3,-13,+15,-16	Abnormal
			6	24,X,+1	Abnormal
			7	28,X,+4,+11,+13,+14,+21	Abnormal
Case 44 (con-tinue)			8	23,X,-2,-14,+18,+21	Abnormal
case 45	45,XX,(13;14)(q10;q10)	TE	1	45,XY,-17	Abnormal
			2	44,XY,-6,-18	Abnormal
			3	46,X0,+13	Abnormal
case 46	46,XX,t(4;11)(q24;q11) [chromosome 4 fragment: 89 Mbp; chromosome 11 fragment: 81 Mbp]	Bla	1	45,XY,-1,-4q25-q35.2+11q13.5-q25,+15,-22	Abnormal
			1	46,XY,+2p14-2q37.3,+3p26.4-q25.3,+4q25-q35.2,+5,-11p11.2-q25,+16p13.3-p11.2,-21	Abnormal
			2	48,XX,+1,-2,-4p16.3-q25,+11,+12,-15,+17,+18,-21	Abnormal
			3	46,XX,+9,+11,-16,-17	Abnormal
			4	44,XX,+3,-4p16.3-q25,+11p15.5-p11.2,-12,-16,-18	Abnormal
case 47	46,XX,inv(5)(p13.1-q13.3) [Pericentric inversion: 37 Mbp Short arm fragment (5pter–5p12): 42 Mbp; Long arm frag-ment (5q13.3–5qter): 108 Mbp]	PB	1	24,X,+X	Abnormal
			2	23,X	Normal/Balanced
			3	23,X	Normal/Balanced
			4	22,X,-18	Abnormal
			5	29,X,+5p15.3-p12,+1,+6,+10,-13,+16,+18,+21,+X	Abnormal
			6	24,X,-5p15.3-p12,+5q11.1-q35.3,+16	Abnormal
			7	24,X,+21	Abnormal
case 48	46,XX,inv(5)(p13.1-q13.3) [Pericentric inversion: 37 Mbp Short arm fragment (5pter–5p12): 42 Mbp; Long arm frag-ment (5q13.3–5qter): 108 Mbp]	TE	1	47,XX,+6	Abnormal
			2	47,XX,+16	Abnormal

Inv: Inversion. PB: polar bodies biopsied from oocytes. Bla: blastomere biopsied from embryos at the cleavage stage. TE: trophectoderm cells biopsied from embryos at the blastocyst stage.

Appendix D

Table D-1: Summary of cases at the cleavage stage for patients with advanced maternal age

Patient number	Maternal age	Embryos examined	Normal	Abnormal	Undiagnosed	Outcome
1	35	2	1	1	0	Not known
2	37	3	1	2	0	Not pregnant
3	37	5	1	4	0	Not pregnant
4 (2 cycles)	37	34	4	30	0	Not pregnant after 1st cycle / no transfer after second cycle
5	37	8	1	7	0	Not known
6	38	6	1	4	1	Not known
7	38	9	0	8	1	No transfer
8	38	8	5	3	0	Pregnant
9	38	10	3	3	4	Not known
10	39	6	1	4	1	Not pregnant
11	39	4	0	2	2	No transfer
12	39	2	0	2	0	No transfer
13	39	7	3	1	3	Pregnant
14	39	5	0	4	1	No transfer
15	39	7	2	3	2	Not known
16	39	6	2	4	0	Not known
17	40	9	0	9	0	No transfer
18	40	2	0	2	0	No transfer
19	40	7	0	7	0	No transfer
20	40	8	1	6	1	Not pregnant
21	40	4	1	1	2	Not known
22	40	14	2	11	1	Pregnant
23	40	8	0	5	3	No transfer
24	40	21	4	17	0	Not pregnant
25	40	5	0	3	2	No transfer
26	40	10	0	8	2	No transfer
27	40	1	0	1	0	No transfer
28	40	1	0	1	0	No transfer
29	40	4	0	4	0	No transfer
30	41	5	0	5	0	No transfer
31	41	5	0	5	0	No transfer

32	41	3	0	3	0	No transfer
33	41	8	0	8	0	No transfer
34	41	7	0	3	4	No transfer
35	41	5	1	4	0	Not known
36	41	8	0	6	2	No transfer
37	41	5	0	4	1	No transfer
38	41	7	1	6	0	Not known
39	41	4	0	4	0	No transfer
40	41	4	0	2	2	No transfer
41	41	8	0	8	0	No transfer
42	41	6	0	3	3	No transfer
43	41	8	1	6	1	Not known
44	41	4	0	3	1	No transfer
45	41	7	0	7	0	No transfer
46	42	5	0	4	1	No transfer
47	42	4	0	4	0	No transfer
48	42	9	1	5	3	Not pregnant
49	42	11	0	7	4	No transfer
50	42	3	0	3	0	No transfer
51	42	7	1	6	0	Not pregnant
52	42	5	1	4	0	Not pregnant
53	42	6	0	6	0	No transfer
54	42	4	0	3	1	No transfer
55	42	5	1	4	0	Not known
56	42	2	1	1	0	Pregnant
57	42	8	0	6	2	No transfer
58	42	9	1	5	3	Not known
59	42	2	0	1	1	No transfer
60	42	5	0	5	0	No transfer
61	43	6	0	4	2	No transfer
62	43	4	0	4	0	No transfer
63	43	5	2	2	1	Not known
64	43	12	1	9	2	Not pregnant
65	43	8	0	6	2	No transfer

66	44	3	0	2	1	No transfer
67	44	7	1	6	0	Not known
68	44	6	0	6	0	No transfer
69	44	9	1	8	0	Not known
70	44	8	0	7	1	No transfer
71	44	7	0	7	0	No transfer
72	44	11	1	10	0	Pregnant
73	45	9	2	7	0	Not known

Table D-2: Summary of cases at the blastocyst stage for patients with advanced maternal age

Patient number	Maternal age	Embryos examined	Normal	Abnormal	Undiagnosed	Outcome
74	35	6	1	4	1	Not pregnant
75	35	8	5	3	0	Pregnant/ twins
76	36	5	3	2	0	Pregnant
77	36	1	1	0	0	Not known
78	36	4	2	2	0	Not known
79	37	2	2	0	0	Not known
80	37	5	2	3	0	Not known
81	37	4	1	2	1	Not known
82	37	9	5	4	0	Not known
83	37	9	5	4	0	Pregnant
84	37	4	2	2	0	Pregnant/ singleton
85	37	4	2	1	1	Not pregnant
86	37	5	4	1	0	Not known
87	37	6	3	3	0	Not known
88	37	3	2	1	0	Pregnant/ singleton
89	38	3	1	2	0	Not known
90	38	5	3	2	0	Not known
91	38	7	4	3	0	Not known
92	38	3	2	1	0	Not known
93	38	6	3	3	0	Twin pregnancy
94	38	6	2	4	0	Pregnant/ singleton
95	38	5	2	3	0	Pregnant
96	39	8	4	3	1	Pregnant/ singleton
97	39	17	5	12	0	Not known
98	39	6	1	5	0	Not known
99	39	8	2	6	0	Not known
100	39	8	6	2	0	Not known
101	39	3	1	2	0	Not known
102	39	1	0	1	0	No transfer
103	39	2	0	1	1	No transfer
104	39	1	1	0	0	No transfer
105	39	1	0	1	0	No transfer

106	39	2	0	2	0	No transfer
107	40	6	2	4	0	Pregnant / singleton
108	40	8	4	4	0	Pregnant, twins
109	40	10	4	2	4	Pregnant / triplets
110	40	15	5	10	0	Not pregnant
111	40	7	3	3	1	Pregnant
112	40	5	2	3	0	Pregnant/ singleton
113	40	7	3	4	0	Not known
114	40	2	0	2	0	No transfer
115	40	3	2	1	0	Not known
116	40	14	5	7	2	Not known
117	40	1	1	0	0	Not known
118	40	5	2	3	0	Not known
119	40	2	1	1	0	Not pregnant
120	40	7	0	7	0	No transfer
121	40	6	2	4	0	Pregnant/ singleton
122	40	3	1	2	0	Not known
123	40	7	3	4	0	Not known
124	40	8	2	5	1	Not known
125	40	3	0	2	1	No transfer
126	40	1	0	1	0	No transfer
127	41	11	4	7	0	Not known
128	41	5	2	2	1	Not pregnant
129	41	5	2	3	0	Not pregnant
130	41	9	3	6	0	Pregnant/ twins
131	41	2	1	1	0	Not known
132	41	7	3	4	0	Pregnant/ singleton
133	41	5	3	2	0	Pregnant/ twins
134	41	8	4	3	1	Pregnant after SET
135	41	7	0	7	0	No transfer
136	41	4	2	2	0	Pregnant, singleton
137	41	5	2	3	0	Not known
138	41	2	0	2	0	No transfer
139	41	3	1	1	1	Not known

140	41	3	1	1	1	Not known
141	41	3	1	2	0	not pregnant
142	41	4	1	3	0	pregnant
143	41	1	0	1	0	No transfer
144	41	3	1	2	0	Not known
145	41	3	0	3	0	No transfer
146	42	2	0	2	0	No transfer
147	42	8	2	4	2	Not known
148	42	7	3	4	0	Pregnant / twins
149	42	1	0	1	0	No transfer
150	42	16	4	7	5	Not known
151	42	4	3	0	1	Pregnant / twins
152	42	4	0	4	0	No transfer
153	42	3	2	1	0	Not known
154	42	3	1	2	0	Not known
155	42	3	1	2	0	Not known
156	42	2	2	0	0	Not known
157	42	8	1	5	2	Not known
158	42	4	1	2	1	Not known
159	42	1	0	1	0	No transfer
160	42	1	0	1	0	No transfer
161	43	6	2	3	1	Not known
162	43	8	0	8	0	No transfer
163	43	3	1	2	0	Pregnant/ singleton
164	43	6	0	6	0	No transfer
165	43	5	1	1	3	Biochemical
166	43	6	0	6	0	No transfer
167	43	6	1	5	0	Not known
168	43	2	1	1	0	Not pregnant
169	43	3	0	3	0	No transfer
170	44	3	1	2	0	Not pregnant
171	44	1	0	1	0	No transfer
172	44	9	3	5	1	Pregnant/ twins
173	44	3	0	3	0	No transfer

174	44	1	0	1	0	No transfer
175	45	2	0	2	0	No transfer
176 (2 cycles)	46	9	1	6	2	No transfer after first cycle/ Not pregnant after second
177	47	3	0	3	0	No transfer

Table D-3: Summary of cases at the cleavage stage for patients with recurrent miscarriage

Patient number	Maternal age	Embryos examined	Normal	Abnormal	Undiagnosed	Outcome
178	31	4	2	1	1	Pregnant
179	34	11	2	9	0	Not pregnant
180	34	16	4	9	3	Not known
181	34	3	2	1	0	Pregnant with monozygotic twins after transfer of 1 embryo
182	34	3	2	1	0	Pregnant / biochemical
183	35	6	2	4	0	Not known
184	35	4	3	1	0	Not known
185	35	8	3	3	2	Not known
186	36	10	3	7	0	Not pregnant
187	37	2	0	2	0	No transfer
188	38	1	0	1	0	No transfer
189	38	14	4	9	1	Pregnant / Twins
190	38	3	1	2	0	Not known
191	38	13	2	11	0	Not known
192	39	5	0	5	0	No transfer
193	39	8	2	4	2	Pregnant
194	40	7	4	2	1	No transfer/ embryos arrested
195	41	4	0	4	0	No transfer
196	42	11	3	8	0	Delivered / Twins
197	43	4	0	4	0	No transfer
198	43	7	0	4	3	No transfer
199	43	8	1	5	2	Not known
200	45	8	1	7	0	No transfer

Table D-4: Summary of cases at the blastocyst stage for patients with recurrent miscarriage

Patient number	Maternal age	Embryos examined	Normal	Abnormal	Undiagnosed	Outcome
201	30	4	1	2	1	Pregnant/ singleton
202	30	8	7	1	0	Not known
203 (2 cycles)	30	13	8	5	0	Pregnant and Miscarried for both cycles
204	30	9	6	3	0	Pregnant
205	31	10	5	4	1	Pregnant / miscarried
206	31	11	8	3	0	Not known
207	31	8	5	1	2	Pregnant after fresh day-6 transfer
208	32	11	3	8	0	Not known
209	32	6	3	2	1	Pregnant / miscarried
210	33	11	3	8	0	Pregnant/ twins
211	33	6	6	0	0	Not known
212	34	15	5	7	3	Pregnant/ twins
213	34	4	2	1	1	Pregnant/ singleton
214	34	5	2	3	0	Not known
215	35	26	10	16	0	Not known
216	35	5	3	2	0	Pregnant after fresh transfer
217	35	6	3	2	1	Pregnant / twins
218	36	9	4	3	2	Pregnant
219	36	4	2	2	0	Not known
220	36	15	6	9	0	Not known
221	36	6	4	1	1	Not known
222	37	5	3	2	0	Pregnant
223	38	12	4	4	4	Pregnant / twins
224	38	3	0	3	0	No transfer
225	38	5	3	2	0	Pregnant
226	38	3	2	1	0	Pregnant
227	39	14	7	7	0	Pregnant / twins
228	40	9	1	8	0	Not known
229	40	6	1	4	1	Pregnant
230	40	8	3	4	1	Pregnant/ delivered twins
231	40	8	4	4	0	No transfer as normal embryos arrested

232	40	2	0	2	0	No transfer
233	41	8	1	6	1	Pregnant
234	41	4	1	3	0	Pregnant
235	41	8	3	5	0	Not known
236	41	1	0	1	0	No transfer
237	42	7	2	4	1	Pregnant / miscarried
238	42	8	3	5	0	Not known
239	43	3	1	1	1	Not pregnant
240	43	1	1	0	0	No transfer as normal embryos arrested
241	43	5	0	5	0	No transfer

Table D-5: Summary of cases at the cleavage stage for patients with repeated IVF failure

Patient number	Maternal age	Embryos examined	Normal	Abnormal	Undiagnosed	Outcome
242	27	7	1	5	1	Not known
243	30	13	3	9	1	Pregnant
244	31	7	3	4	0	Not pregnant
245	34	6	3	3	0	Pregnant
246	34	6	0	6	0	No transfer
247	35	4	1	3	0	Not known
248	35	4	1	3	0	Not known
249	36	2	0	1	1	No transfer
250 (2 cycles)	37	15	1	13	1	No transfer after first cycle/ Not known after second
251	37	3	0	1	2	No transfer
252	38	6	2	4	0	Not pregnant
253	39	7	4	3	0	Pregnant / twins
254	39	9	2	7	0	Not known
255	40	6	0	6	0	No transfer
256	42	5	0	4	1	No transfer
257	42	22	2	16	4	Not pregnant
258	42	15	0	12	3	No transfer
259	44	7	1	6	0	Not known

Table D-6: Summary of cases at the cleavage stage for patients with repeated IVF failure

Patient number	Maternal age	Embryos examined	Normal	Abnormal	Undiagnosed	Outcome
260	28	8	7	1	0	Not known
261	29	7	4	3	0	Pregnant/ singleton
262	32	9	5	3	1	Pregnant/ singleton
263	32	19	10	8	1	Not known
264	33	5	3	2	0	Pregnant/twins
265	34	4	0	4	0	No transfer
266	34	16	12	4	0	Not pregnant
267	34	2	1	0	1	Not known
268	35	5	3	2	0	Not known
269	36	4	4	0	0	Pregnant/ triplets
270	36	12	2	9	1	Pregnant/ singleton
271	36	9	2	6	1	Biochemical
272	36	4	1	3	0	Not known
273	36	6	4	2	0	Not known
274	36	5	1	3	1	Not known
275	36	2	0	2	0	No transfer
276	37	10	3	7	0	Pregnant with twins
277	37	9	6	3	0	Pregnant/ Ectopic
278	37	5	2	3	0	Pregnant/twins
279	37	8	3	5	0	Pregnant
280	37	5	2	3	0	Not known
281	37	3	0	3	0	No transfer
282	38	6	4	1	1	Pregnant with twins
283	38	5	2	3	0	Not known
284	39	12	6	6	0	Not known
285	39	8	3	4	1	Pregnant
286	40	2	1	1	0	Not known
287	40	5	3	2	0	Pregnant/ singleton
288 (2 cycles)	40	7	2	5	0	Not pregnant after 1st cycle and no transfer after 2nd
289	40	3	1	2	0	Not known
290	41	5	2	1	2	Not known

291	41	5	1	3	1	Pregnant
292	41	3	0	3	0	No transfer
293	41	12	4	8	0	Pregnant/ singleton
294	41	8	3	4	1	Pregnant from fresh cycle
295	42	2	1	1	0	Not pregnant
296	42	13	5	8	0	Not known
297	42	2	0	2	0	No transfer
298	42	3	2	1	0	Pregnant with twins
299	43	3	0	3	0	No transfer
300	44	4	0	3	1	No transfer

Table D-7: Cytogenetic results for embryos at the cleavage stage from patients at advanced maternal age

Patient	Maternal age	Embryo number	Embryos karyotype	Embryo characterisation
1	35	6	42,XX,-2,-8,-11,-20	Abnormal
	35	8	46,XX	Normal
2	37	1	46,XX,-7q11.21-qter	Abnormal
	37	2	44,XY,-1,-4,-5pter-5p12,+5q11.1-qter	Abnormal
	37	3	46,XX	Normal
3	37	1	45,XX,-16	Abnormal
	37	3	45,XY,-13	Abnormal
	37	4	46,XY	Normal
	37	7	47,XY,-9q,+14	Abnormal
	37	9	45,XY,-8	Abnormal
4 (1st)	37	1	47,XY,+16	Abnormal
	37	2	47,XX,+7	Abnormal
	37	3	46,XX	Normal
	37	4	43,XY,-1,-6,+14,-15,+17,-21,-22	Abnormal
	37	5	47,XX,+6	Abnormal
	37	6	46,XY,+15,-17	Abnormal
	37	7	48,XXY,+19	Abnormal
	37	8	45,XX,-19	Abnormal
	37	9	47,XXY,-1,-13	Abnormal
	37	10	47,XY,+22	Abnormal
	37	11	47,XX,+8	Abnormal
	37	12	46,XY,+9q,-14,-16,+22	Abnormal
	37	13	39,X0,-12,-13,-16,-17,-19,-21	Abnormal
	37	14	41,XX,+1p,-3,-9,-13,-17,-22	Abnormal
	37	15	44,XY,-8,-16	Abnormal
	37	16	45,XX,-13	Abnormal
	37	17	46,XX,+14,-15	Abnormal
	37	18	47,XY,+22	Abnormal
	37	19	48,XX,+15,+21	Abnormal
	37	20	46,XY,-1,+3,-5,-10,+11,+14	Abnormal
4 (2nd)	37	1	48,XX,-4,+14,+17,+19,+21,-22	Abnormal

	37	2	46,XY	Normal
	37	3	45,XX,-16	Abnormal
	37	4	68,XXY,--16	Abnormal
	37	5	47,XX,+2p25.3_2p13.3,+22	Abnormal
	37	6	46,XY	Normal
	37	7	47,XX,+9	Abnormal
	37	8	69,XXY	Abnormal
	37	9	52,XXY,+1,+5,+10,+11,-12,+13,+15,+17,-18,+19,-21	Abnormal
	37	10	47,XXY,+19,-22	Abnormal
	37	11	45,XY,-2p25.3_2p11.2,-9q,+15,-18,-22	Abnormal
	37	12	46,XX	Normal
	37	13	48,XY,-2p25.3_2p11.2,+4,+22	Abnormal
	37	14	46,XY,-16,+19	Abnormal
5	37	1	45,XX,-15	Abnormal
	37	2	45,XX,-20	Abnormal
	37	3	48,XY,+11,+17	Abnormal
	37	4	45,XY,-13	Abnormal
	37	5	44,X,+1,-2,+3,-4,+5,-6,-7,-8,-9,+10,+11,+12,+13,+14,-15,-16,-17,-18,+19,-20,+21,-22	Abnormal
	37	6	45,XX,+2,-6,+9,-10,-12	Abnormal
	37	7	45,XX,-11	Abnormal
	37	8	46,XY	Normal
6	38	2	50,XX,+4,+5,+11,+12	Abnormal
	38	3	50,XX,+1,+15,+16,+18	Abnormal
	38	4	46,XX	Normal
	38	5	45,XX,-14,-16,+22	Abnormal
	38	6	No result	Undiagnosed
	38	7	45,XX,-11	Abnormal
7	38	1	36,-2,-3,-10,-11,-17,-18,+19,-20,-21,-22,-X,-Y	Abnormal
	38	2	44,XX,-4,-9	Abnormal
	38	4	No result	Undiagnosed
	38	5	47,XX,+16	Abnormal
	38	6	44,X0,-4,+13,-22	Abnormal
	38	9	44,XY,-8,-11	Abnormal

	38	11	49,Y0,+2,-3,+5,-7,+8,-10,+11,-12,+14,-15,+17,+18,+19,+22,-X	Abnormal
	38	14	46,XX,+8p,-8q	Abnormal
	38	15	50,+3,+6,+7,+8,+9,+10,-19,+22,-X,Y	Abnormal
8	38	1	45,XY,-4	Abnormal
	38	2	46,XX	Normal
	38	3	46,XX	Normal
	38	4	45,XY,-22	Abnormal
	38	5	45,XX,-19	Abnormal
	38	6	46.XX	Normal
	38	7	46,XY	Normal
	38	8	46,XY	Normal
9	38	1	No result	Undiagnosed
	38	2	No result	Undiagnosed
	38	3	46,XY	Normal
	38	4	47,XY,+21	Abnormal
	38	5	46,XY	Normal
	38	7	45,XX,-8	Abnormal
	38	8	46,XY	Normal
	38	9	No result	Undiagnosed
	38	10	46,XY,-16,+21	Abnormal
	38	11	No result	Undiagnosed
10	39	1	46,XY	Normal
	39	2	45,XX,-15	Abnormal
	39	3	47,XX,+13	Abnormal
	39	4	47,Y0,-6,-8,+12,+13,+15,+18,+19,-21,-X	Abnormal
	39	5	46,XX,+12q	Abnormal
	39	6	No result	Undiagnosed
11	39	5	No result	Undiagnosed
	39	6	No result	Undiagnosed
	39	8	45,XX,-15	Abnormal
	39	9	43,XX,+3,-4,+10q,-16,-18,-20	Abnormal
12	39	2	48,XX,+2,+4,+5,-6,-7,-8,+9,-10,-11,-13,+15,+16,+17,+20,+22	Abnormal
	39	9	46,XY,+16,-18	Abnormal
13	39	1	45,XX,+18p,-22	Abnormal

	39	2	46,XY	Normal
	39	3	No result	Undiagnosed
	39	4	46,XX	Normal
	39	5	46,XY	Normal
	39	6	No result	Undiagnosed
	39	7	No result	Undiagnosed
14	39	1	45,X0,-12	Abnormal
	39	2	No result	Undiagnosed
	39	3	45,XX,-1	Abnormal
	39	4	47,XX,+1	Abnormal
	39	5	47,XY,+9	Abnormal
15	39	1	46,XX	Normal
	39	2	48,XY,+12,+15	Abnormal
	39	3	47,XX,+11	Abnormal
	39	4	47,XY,-7q31.32_7q36.3,+16	Abnormal
	39	5	No result	Undiagnosed
	39	6	46,XX	Normal
	39	7	No result	Undiagnosed
16	39	5	46,XX	Normal
	39	6	46,XY,-11,+19	Abnormal
	39	7	47,XY,+19	Abnormal
	39	8	46,XY,+16,-18	Abnormal
	39	11	47,XX,+1q21.1_1q44	Abnormal
	39	12	46,XY	Normal
17	40	1	45,XX,-14	Abnormal
	40	2	47,XX,+8,-15,+16	Abnormal
	40	3	47,XX,+22	Abnormal
	40	4	45,XY,-11	Abnormal
	40	5	47,XY,+15	Abnormal
	40	6	48,XY,+15,+21	Abnormal
	40	7	47,XY,+10	Abnormal
	40	8	42,XX,-14,-15,-20,-21	Abnormal
	40	9	43,X0,+6,-10,-14,-22	Abnormal
18	40	1	48,Y0,-2,-5,+12,+17,+18,+22	Abnormal

	40	2	49,XX,+1,+2,-4p,+7,+10,-11,-12,+13,-14,-16,+17,+18,-19,+22	Abnormal
19	40	3	47,XY,-11q,+22	Abnormal
	40	4	45,XX,-13	Abnormal
	40	5	46,X0,+17	Abnormal
	40	6	45,XX,-22	Abnormal
	40	8	45,XX,-2,-16q	Abnormal
	40	9	46,XY,-8p,+8q	Abnormal
	40	10	42,XY,+3,-4,+12,-14,-16,-17,-18,-20	Abnormal
20	40	1	48,XY,+1,+2q,+9	Abnormal
	40	2	No result	Undiagnosed
	40	3	46,XX,+13,-15	Abnormal
	40	4	45,XY,-19	Abnormal
	40	5	40,XX,-2,+5,-6,-8,-12,-19,-20,-21	Abnormal
	40	6	46,XY	Normal
	40	7	43,XX,-12,-15,-16	Abnormal
	40	8	50,XX,-1,+2,+3,+5,-8,+10,+12,-13,+16.+17,+18,-20,+21,-22	Abnormal
21	40	1	No result	Undiagnosed
	40	2	No result	Undiagnosed
	40	3	46,XX	Normal
	40	4	46,XX,-4q28.3_q35.2,-13,+21	Abnormal
22	40	1	49,XX,+14,+15,+21	Abnormal
	40	2	47,XX,+22	Abnormal
	40	3	46,XY	Normal
	40	4	45,XX,-22	Abnormal
	40	5	45,XY,-15	Abnormal
	40	6	47,XY,+13	Abnormal
	40	7	No result	Undiagnosed
	40	8	45,XY,-19	Abnormal
	40	9	49,Y0,+4,-6,+8,+10,+14,+16,+17,-18,+19,-22	Abnormal
	40	10	53,XXY,+1,+3,+5,-8,+9,+12,+14,-15,-16,+18,+19	Abnormal
	40	11	45,Y0,-7,+12,-14,+16,-22,-X	Abnormal
	40	12	45,XY,+1,+2,-8,-9,+10,+11,+12,+13,-14,-15,+16,-18,-20,-22	Abnormal
	40	13	45,XX,-16	Abnormal
	40	14	46,XX	Normal

23	40	1	44,XX,-1,-5,+15,-18	Abnormal
	40	2	48,XX,+18,+19	Abnormal
	40	3	No result	Undiagnosed
	40	4	47,XX,+8,+9,-15	Abnormal
	40	5	No result	Undiagnosed
	40	6	47,XY,-1,+2,+9,-18,-20,+21,+22	Abnormal
	40	7	No result	Undiagnosed
	40	8	48,XXY,-3,-4,-5,-8p,+10,+13,-14,+15,+18,-20,+21,	Abnormal
24	40	1	47,XX,+17	Abnormal
	40	2	48,XXY,+3,+8,-18	Abnormal
	40	3	45,XX,+17p,-22	Abnormal
	40	4	41,X0,-2,-3,-14,-15	Abnormal
	40	5	46,XY	Normal
	40	6	46,XX	Normal
	40	7	46,XY	Normal
	40	8	49,XY,+2,+3,+10	Abnormal
	40	9	47,XX,+14	Abnormal
	40	10	44,XY,-15,-18	Abnormal
	40	11	46,XY	Normal
	40	12	46,XX,+9,-22	Abnormal
	40	13	44,XX,+4q,-15,-21	Abnormal
	40	14	45,XY,-15	Abnormal
	40	15	69,XXY, triploid	Abnormal
	40	16	47,XY,+18	Abnormal
	40	17	47,XX,+7,+10,-22	Abnormal
	40	18	47,XY,+13,+15	Abnormal
	40	19	47,XX,+2	Abnormal
	40	20	48,XX,+15,+16	Abnormal
	40	21	47,XY,+9,+15	Abnormal
25	40	1	No result	Undiagnosed
	40	6	No result	Undiagnosed
	40	8	39,XX,-1,+2,-13,-14,-16,-17,-18,-19,-20,-21,+22	Abnormal
	40	14	49,XX,+6,+13,+19	Abnormal
	40	18	46,XY,-1p36.33_1p36.32	Abnormal

26	40	1	No result	Undiagnosed
	40	2	53,XX,+5,-6,+7,+10,+15,+18,+19,+20,+21	Abnormal
	40	3	No result	Undiagnosed
	40	4	45,XY,-16	Abnormal
	40	5	44,XY,-10,-18,+19,-21	Abnormal
	40	6	44,XXY,-13,-15,-22	Abnormal
	40	7	45,XX,-22	Abnormal
	40	8	51,XX,+2,+3,+10,+11,+12,+16,-20	Abnormal
	40	9	47,XX,+21	Abnormal
	40	10	46,XX,-4,+22	Abnormal
27	40	1	47,XY,+20	Abnormal
28	40	1	46,XX,-9,+16,+17,+18,-19,-22	Abnormal
29	40	1	44,XY,-16,-22	Abnormal
	40	2	46,XX,-2,+15	Abnormal
	40	4	47,XXY,+Xq22.1_Xq28	Abnormal
	40	5	47,XX,+21	Abnormal
30	41	1	46,XX,+2,+9q,-15,+18,-21	Abnormal
	41	2	44,Y0,-16,-X	Abnormal
	41	3	47,XY,+1q,-4,-5,-6,+8,-9,-12,+13,+14,+15,+16,-20	Abnormal
	41	5	46,XX,-4,+18,-21,+22	Abnormal
	41	6	47,XX,+22	Abnormal
31	41	1	45,XX,+9,-12,-13,+15,+16,-17,+18,-19,-22	Abnormal
	41	2	44,X,-6,-10	Abnormal
	41	3	47,XX,+13	Abnormal
	41	4	51,X0,+5,+7,+14,+16,+20,+22	Abnormal
	41	5	41,XX,-1,-5,-13,-14,+15,-21,-22	Abnormal
32	41	2	45,XX,-2,-7,+8	Abnormal
	41	5	43,XY,-10,-15,-19	Abnormal
	41	8	46,XY,-5,+12,-15,+19	Abnormal
33	41	1	48,XX,+10,+16,+17,-19,-21,+22	Abnormal
	41	2	43,XY,-2,-5p,-7,+8q,+9,-21,-22	Abnormal
	41	3	46,XY,+21,-22	Abnormal
	41	6	44,X0,-2q35_2q37,-14	Abnormal
	41	7	48,XY,+15,+22	Abnormal

	41	8	46,XX,+12p13.33_12q21.33,-16,+21	Abnormal
	41	9	45,X0	Abnormal
	41	8	45,XX,-19	Abnormal
34	41	1	No result	Undiagnosed
	41	7	No result	Undiagnosed
	41	10	No result	Undiagnosed
	41	14	No result	Undiagnosed
	41	15	44,XY,-2,-17	Abnormal
	41	19	45,XX,-1,-11q13.2_q25,+14,-22	Abnormal
	41	20	45,XX,+9,-15,-16	Abnormal
35	41	1	47,XX,+19	Abnormal
	41	2	48,XX,-1,+2,+6,+7,+8,+9,-12,-15,+20,-21	Abnormal
	41	3	46,XY	Normal
	41	4	47,XY,+16	Abnormal
	41	5	45,XX,-3q,-22	Abnormal
36	41	1	47,XXY,-12,+19	Abnormal
	41	2	47,XY,-1,+2,-3,+6,+7,+12,-15,-19,+22	Abnormal
	41	3	49,X0,+5,+6,+7,+9,+10,-12,+13,-15,+16,-17,+18,-22	Abnormal
	41	4	45,XXYY,+1,-2,-4,-8,-9,+10,+12,-13,+14,+15,-16,-17,-19,+20	Abnormal
	41	5	No result	Undiagnosed
	41	6	No result	Undiagnosed
	41	7	45,XY,-8	Abnormal
	41	8	50,XX,+1,+3,+5,+7,-10,-12,+14,-15,+16,+17,-19,+20	Abnormal
37	41	1	51,XX,+1,-2,+5,+7,+8,-16,+17,-18,+20,+21,+22	Abnormal
	41	2	46,XY,+2,-15,+16,-22	Abnormal
	41	3	48,XY,+21,+22	Abnormal
	41	4	46,XX,-1,+2,+3,+4,-5,+6,+18,-20,-21,22	Abnormal
	41	5	No result	Undiagnosed
38	41	1	49,X0,+3,+17,+18p/q,+21	Abnormal
	41	2	44,XY,+1,-3,-9p,+14,-16p,-17,-21,-22	Abnormal
	41	3	44,XY,-2,-5,+19,-20	Abnormal
	41	4	46,XX	Normal
	41	5	45,XX,-1,-8p,+12q	Abnormal
	41	6	46,X0,+1q,+20	Abnormal

	41	7	46,XX,-15,+16	Abnormal
39	41	1	46,XY,-1,-4,+15,+21	Abnormal
	41	2	42,X0,-1,-3,-6q,-10,+12,+13,+14,+15,-18,-21	Abnormal
	41	3	47,XXY,+9p	Abnormal
	41	4	46,XX,+1,-21	Abnormal
40	41	1	No result	Undiagnosed
	41	2	39,XX,-2,-5,-7,+12,-14,-17,-20,-21,-22	Abnormal
	41	3	No result	Undiagnosed
	41	4	45,XY,-4,+8,-10	Abnormal
41	41	5	45,XY,-9	Abnormal
	41	6	49,XX,+1,+5,+7,-9,+19	Abnormal
	41	7	46,XX,-1q,-4,+18	Abnormal
	41	9	45,XX,-11p,-15	Abnormal
	41	12	46,XX,+5,-7,-18q	Abnormal
	41	15	47,XY,-2p,+2q,+15	Abnormal
	41	16	45,XX,-21	Abnormal
	41	17	44,XY,-8p,-15,-18	Abnormal
42	41	1	47,XY,+20	Abnormal
	41	2	No result	Undiagnosed
	41	3	45,XX,-2,-5,+9,+11,-12,+15,-16,-19,+21	Abnormal
	41	5	No result	Undiagnosed
	41	6	45,XX,-1,-4,+21	Abnormal
	41	7	No result	Undiagnosed
43	41	2	69,XXY	Abnormal
	41	3	45,XX,-19	Abnormal
	41	4	47,XY,+19	Abnormal
	41	6	47,XX,-2,+9,+19	Abnormal
	41	10	46,XX,+19,-20	Abnormal
	41	11	46,XX	Normal
	41	12	46,XY,+2,-19	Abnormal
	41	13	No result	Undiagnosed
44	41	1	47,XX,+16,+19	Abnormal
	41	2	47,XY,+16	Abnormal
	41	3	45,XX,-18	Abnormal

	41	4	No result	Undiagnosed
45	41	2	48,XY,-7,+15,+19	Abnormal
	41	6	45,XY,-13	Abnormal
	41	7	49,XX,+9,+14,+16,+17,-21	Abnormal
	41	8	47,XX,+12	Abnormal
	41	12	53,XXY,+1,+3,+6,+8,+16,+19	Abnormal
	41	13	47,XX,+10	Abnormal
	41	14	43,XY,-3,-5,-16,-22	Abnormal
46	42	1	46,XX,+6,-8,-14,+16	Abnormal
	42	2	46,XY,-18q	Abnormal
	42	3	38,XX,-5,-7,-10,+14,-15,-16,-17,-18,-21,-22	Abnormal
	42	4	No result	Undiagnosed
	42	5	49,X0,-1,-3.+14,+16,+18,+20,+22	Abnormal
47	42	1	45,X0	Abnormal
	42	7	45,X0	Abnormal
	42	9	41,XX,-6,-7,-13,-14,-17,-19,+22	Abnormal
	42	10	44,XY,-8,-22	Abnormal
48	42	1	44,X,-14,-15	Abnormal
	42	2	No result	Undiagnosed
	42	3	46,XY	Normal
	42	4	46,XX,-11,+21	Abnormal
	42	5	No result	Undiagnosed
	42	6	48,XX,+5,+10	Abnormal
	42	7	No result	Undiagnosed
	42	8	47,XY,+7,+19p	Abnormal
	42	9	48,XX,+1,+2,+8,+9,-10,-11,-12,-14,+17,+21	Abnormal
49	42	1	45,XX,+11,-15,-19q,-21	Abnormal
	42	2	49,XX,+13,+16,+17	Abnormal
	42	3	46,XY,+3,-5,-7,+8,-10,+13,+19,-21	Abnormal
	42	4	No result	Undiagnosed
	42	5	No result	Undiagnosed
	42	6	No result	Undiagnosed
	42	7	43,XX,-4,-5,-16	Abnormal
	42	8	No result	Undiagnosed

	42	9	44,XX,+5,-9,-13,-14,+19,-21,+Xq	Abnormal
	42	10	48,XY,+4,+21	Abnormal
	42	11	44,XY,-7,-22	Abnormal
50	42	3	45,X0,-5,-6,-8,+12,+16,-17,+19,+22	Abnormal
	42	4	46,XX,-5,+14,+15,+19,-21,-22	Abnormal
	42	5	48,XY,+2,-4,-9,+13,-14,+15,+16,-19,+20,+21	Abnormal
51	42	1	46,XX	Normal
	42	4	45,XY,-9	Abnormal
	42	5	44,XX,-4,-19	Abnormal
	42	6	43,XX,-14,-17,-22	Abnormal
	42	7	45,XX,-4q,-5,+7	Abnormal
	42	9	37,XY,-2,+5,-6,-7,-8,-12,-14,-16,-17,-18,-20	Abnormal
	42	12	53,XX,+3,+6,+8,+9,+17,+19,+22	Abnormal
52	42	1	46,XY	Normal
	42	2	44,XX,-7,-9	Abnormal
	42	3	45,XY,-21	Abnormal
	42	4	45,XX,-22	Abnormal
	42	5	43,XY,-17,19,-22	Abnormal
53	42	3	46,XY,-Xq	Abnormal
	42	5	47,XX,+20	Abnormal
	42	6	46,XX,-14,+21	Abnormal
	42	7	44,XX,-9,-13	Abnormal
	42	11	46,XY,+10,-16	Abnormal
	42	19	45,XY,-19	Abnormal
54	42	1	46,XY,-1,+11,-16,+20,-21,+22	Abnormal
	42	2	41,XX,+4,-7,-10,-11,-15,-18,-21	Abnormal
	42	3	43,XX,-13,-14,-15	Abnormal
	42	4	No result	Undiagnosed
55	42	1	47,XX,+13	Abnormal
	42	3	46,XX	Normal
	42	5	46,XX,-3p,+3q,-10q	Abnormal
	42	7	42,XY,-12,-17,-21,-22	Abnormal
	42	11	46,X0,-22	Abnormal
56	42	1	44,XX,-9,-22	Abnormal

	42	2	46,XX	Normal
57	42	1	47,XX,+2,+3,+4,+6,+7,-10,-13,-14,-20	Abnormal
	42	2	44,XX,+1,-12,-13,-14	Abnormal
	42	3	52,+8,+12,+14,+15,+16,+17,+19,+20,-X,-Y	Abnormal
	42	4	46,XX,-1,+14	Abnormal
	42	5	50,XX,+1,-3,+4,-6,+9,-11,+14,+15,+16,+20,-21,+22	Abnormal
	42	6	No result	Undiagnosed
	42	7	No result	Undiagnosed
	42	8	41,XY,-1,-12,-13,-16,-17,+19,-22	Abnormal
58	42	1	46,XX	Normal
	42	2	46,XY,+2,-22	Abnormal
	42	3	44,XY,-12,-16	Abnormal
	42	4	45,XX,-22	Abnormal
	42	5	45,XY,-20	Abnormal
	42	6	45,XX,-20	Abnormal
	42	7	No result	Undiagnosed
	42	8	No result	Undiagnosed
	42	9	No result	Undiagnosed
59	42	1	45,XY,-2,-5,+9,+10q,+16q,+17p,-18,+20	Abnormal
	42	2	No result	Undiagnosed
60	42	1	47,XX,+21	Abnormal
	42	2	47,XY,+15,+16,-20	Abnormal
	42	3	52,XY,+2,+6,+12,+17,-18q,+19,+22	Abnormal
	42	4	46,XY,+15,-21	Abnormal
	42	5	45,X0,+19p	Abnormal
61	43	1	48.XYY,+6,+7,-8,-9,+13,+14,-15,-16,+20	Abnormal
	43	5	49,XXY,+7,-10,+17,-18,+21,+22	Abnormal
	43	7	48,+1,-2,+6,+7,+10,+14,+16,-18,+21,-22,-X,-Y	Abnormal
	43	8	45,XX,+1,+2,+5,-7,+8,-9,-10,+11,-13,-15,-18,-19,+21,-22	Abnormal
	43	9	No result	Undiagnosed
	43	10	No result	Undiagnosed
62	43	1	47,XX,-2,+16,+17,+21,-22	Abnormal
	43	3	47,XX,+21	Abnormal
	43	5	49,XX,+4,+9,+15	Abnormal

	43	6	45,XX,-15,-16,+19	Abnormal
63	43	1	46,XY	Normal
	43	2	No result	Undiagnosed
	43	3	46,XY,-5,+9,+15,+17,+19q,-20,-22	Abnormal
	43	4	46,XX	Normal
	43	5	47,XX,-7p22.3_7q11.21,+7q11.21_7q36.3,+20	Abnormal
64	43	1	46,XX,-15,+19	Abnormal
	43	3	41,Y0,-1,+10,+11,-14,-15,-16,-17,-22,-X	Abnormal
	43	4	45,XY,-22	Abnormal
	43	5	48,XX,+3,+8,-19,+22	Abnormal
	43	6	46,XX	Normal
	43	7	47,XY,+3,+9p24.3_9q12,-9q21.11_9q34.4,-10,-16q,+19	Abnormal
	43	8	49,XY,+1,+3,-15,+16,+21	Abnormal
	43	9	43,XX,+1,-2,+5,-9,-11,-12,-15,-16,+17,+20	Abnormal
	43	10	37,XY,-2,-5,-8,-11,-12,-13,-14,-17,-18	Abnormal
	43	11	52,XX,-1,+5,+6,+8,+9,+10,+13,-15,+18,+22	Abnormal
	43	12	No result	Undiagnosed
	43	13	No result	Undiagnosed
65	43	1	No result	Undiagnosed
	43	2	46,XX,-14,+15	Abnormal
	43	3	46,XX,-9,+12	Abnormal
	43	4	45,XY,-16	Abnormal
	43	5	47,XY,+22	Abnormal
	43	6	No result	Undiagnosed
	43	7	47,XX,+11,+19,-21	Abnormal
	43	8	46,XY,+14,-16	Abnormal
66	44	1	47,XX,+13	Abnormal
	44	2	47,XX,+13	Abnormal
	44	3	No result	Undiagnosed
67	44	8	46,XY	Normal
	44	9	48,XY,+16,+22	Abnormal
	44	12	48,XX,+13,+17	Abnormal
	44	14	47,XX,+1,+14,+15	Abnormal
	44	15	45,XY,-11	Abnormal

	44	16	44,XX,-9,-13,+16,-20	Abnormal
	44	17	47,XY,-4,+12,+16,+21,-22	Abnormal
68	44	1	45,X0,+2,-17,+18p,+19,-20	Abnormal
	44	2	44,XYY,-2,-3,-7,-9,+12,+14,+15,-16,-17,-18,+22	Abnormal
	44	3	42,XX,-8,-15,-16,-18	Abnormal
	44	4	47,XX,+13,+19p	Abnormal
	44	5	46,XY,+2q,+13,-22	Abnormal
	44	6	48,XX,-10,+15,+19,+22	Abnormal
69	44	1	42,XY,-14,-15,-20,-21	Abnormal
	44	2	42,XX,-7,-8,-9,-15,+18,-22	Abnormal
	44	3	44,X0,-16	Abnormal
	44	4	45,XY,+5,-13,+14,-16,-19	Abnormal
	44	5	51,XXY,+1,+3,+5,-8,-9,-10,+11,+13,-14,+15,+16,+18	Abnormal
	44	6	48,XY,+9,+10	Abnormal
	44	7	45,X0	Abnormal
	44	8	43,XX,-7,-15,-21	Abnormal
	44	9	46,XY	Normal
70	44	1	43,XX,+2,-4,-16,-18,-22	Abnormal
	44	2	52,+2,+5,+6,+9,+10,+11,+13,-18,+19,+20,+21,-22,-X,-Y	Abnormal
	44	3	48,XY,+11,+21	Abnormal
	44	4	47,XY,=7p,-14,+15,+16	Abnormal
	44	5	47,XX,+11,+12,-21	Abnormal
	44	6	43,XX,-15,-16,+20	Abnormal
	44	7	49,XX,+14,+15,+19	Abnormal
	44	8	No result	Undiagnosed
71	44	1	47,XY,+9,-15,+16,+19,-20	Abnormal
	44	2	45,XX,-13,-15,+19	Abnormal
	44	3	48,XX,+15,+18,+20,-21	Abnormal
	44	4	44,XX,-2,-18	Abnormal
	44	5	45,XX,-15	Abnormal
	44	6	44,XY,-7,-12,-16,+19	Abnormal
	44	7	45,XY,-7,+14,-22	Abnormal
72	44	1	45,XX,-8	Abnormal
	44	2	40,XX,-2,-3,-6,-8,-10,-11,-17,+19	Abnormal

	44	3	45,XY,-2,-4,-7,+8,+9,+10,-14,+15,-16,-19,+22	Abnormal
	44	4	46,XY,+13,-16,-19,+21	Abnormal
	44	5	46,XY	Normal
	44	6	44,XY,-15,-18,-21,+22	Abnormal
	44	7	46,XY,-14,+16	Abnormal
	44	8	46,XY,+12,-22	Abnormal
	44	9	45,XY,-13	Abnormal
	44	10	45,XX,+3,-16,-17	Abnormal
	44	11	54,XX,+3,+8,+9,+10,+11,+12,+15,+16,-19,+20	Abnormal
73	45	1	44,-4,+7,-9,-10,+13,+18,-X,-Y	Abnormal
	45	2	46,XXY,-2,+3,+5,+12,-16,-18,+19,-20,-22	Abnormal
	45	3	46,XY,+4,-6,+9,+16,-19,-21	Abnormal
	45	4	46,XY	Normal
	45	5	67,XXY,-1,+8,-10,-16	Abnormal
	45	6	46,XX,-1q,+2p,+7q,+9q,-10,+12q,+14,+16q,+19,-Xq	Abnormal
	45	7	46,XX	Normal
	45	8	45,Y0,-1,-13,+15,+19,-X	Abnormal
	45	9	39,XX,-3,-4,-6,-8,-10,+13,-15,-16,-22	Abnormal

Table D-8: Cytogenetic results for embryos at the blastocyst stage from patients at advanced maternal age

Patient number	Maternal age	Embryo number	CGH result	Embryo characterisation
74	35	1	45,XY,-18	Abnormal
	35	2	45,XY,-16	Abnormal
	35	3	46,XX	Normal
	35	10	45,XX,-16	Abnormal
	35	11	SG failure/ No result	Undiagnosed
	35	13	46,XX,+1q21.1-q44	Abnormal
75	35	1	46,XX	Normal
	35	2	46,XY	Normal
	35	3	46,XX	Normal
	35	4	46,XX	Normal
	35	5	44,-X,-Y	Abnormal
	35	6	45,Y0	Abnormal
	35	7	46,XY	Normal
	35	8	45,Y0	Abnormal
76	36	1	46,XX,-2,+7	Abormal
	36	2	46,XX	Normal
	36	3	46,XX	Normal
	36	4	47,XX,+16	Abnormal
	36	5	46,XX	Normal
77	36	1	46,XY	Normal
78	36	1	47,XX,+22	Abnormal
	36	2	46,XY	Normal
	36	3	46,XY	Normal
	36	4	47,XY,+15,-19p13.3_q13.13	Abnormal
79	37	4	46,XX	Normal
	37	5	46,XX	Normal
80	37	3	45,XY,-18	Abnormal
	37	4	46,XX	Normal
	37	8	47,XY,+22	Abnormal
	37	9	47,XX,+1p,+3,-5q,-14,+15,+16,-21,-22	Abnormal

	37	13	46,XX	Normal
81	37	4	46,XY	Normal
	37	5	46,XX,+16,-21	Abnormal
	37	6	No result	Undiagnosed
	37	7	46,XX,-16,+21	Abnormal
82	37	2	46,XX,+14,-21	Abnormal
	37	3	46,XY	Normal
	37	4	46,XY	Normal
	37	7	46,XY	Normal
	37	9	45,XX,-8	Abnormal
	37	10	45,XY,-22	Abnormal
	37	11	47,XX,+22	Abnormal
	37	12	46,XX	Normal
	37	13	46,XX	Normal
83	37	1	46,XY	Normal
	37	2	46,XY	Normal
	37	7	46,XX	Normal
	37	8	46,XY	Normal
	37	9	47,XX,+19	Abnormal
	37	10	48,XY,+13,+16	Abnormal
	37	11	46,XX	Normal
	37	12	46,XY,-9,+19	Abnormal
	37	13	45,XY,-16	Abnormal
84	37	2	47,XY,+22	Abnormal
	37	6	44,XX,-9,-11,-13,+15	Abnormal
	37	15	46,XX	Normal
	37	20	46,XX	Normal
85	37	1	46,XX	Normal
	37	2	No result	Undiagnosed
	37	3	45,XX,-5	Abnormal
	37	5	46,XY	Normal
86	37	2	46,XX	Normal
	37	4	46,XY	Normal
	37	5	46,XY	Normal

	37	8	46,XX	Normal
	37	10	45,XX,-7	Abnormal
87	37	5	47,XY,+15	Abnormal
	37	6	46,XY	Normal
	37	10	46,XX,+15,-22	Abnormal
	37	14	46,XX	Normal
	37	16	48,XX,+1,+5	Abnormal
	37	19	46,XX	Normal
88	37	5	45,XY,-22	Abnormal
	37	6	46,XY	Normal
	37	8	46,XX	Normal
89	38	6	47,XX,+22	Abnormal
	38	12	46,XX	Normal
	38	13	45,XY,-13	Abnormal
90	38	1	45,XX,-11	Abnormal
	38	2	46,XY	Normal
	38	3	46,XX	Normal
	38	4	45,XY,-11	Abnormal
	38	5	46,XY	Normal
91	38	1	45,XY,-15	Abnormal
	38	3	46,XY	Normal
	38	4	45,XX,-22	Abnormal
	38	6	46,XX	Normal
	38	7	46,XX	Normal
	38	8	47,XX,+11	Abnormal
	38	10	46,XY	Normal
92	38	7	45,XY,-20	Abnormal
	38	12	46,XX	Normal
	38	18	46,XY	Normal
93	38	3	45,XX,-21	Abnormal
	38	4	45,XX,-13	Abnormal
	38	5	45,XX,-1,-2,+8,-10,+11,+14,-15,-16,+17,+18,-20	Abnormal
	38	6	46,XY	Normal
	38	1	47,XY,-4,+21,+22	Abnormal

	38	2	47,XY,-4q(entire arm),+22	Abnormal
94	38	1	46,XY	Normal
	38	9	44,XY,+3,-4,+7,-9,-17,-18	Abnormal
	38	14	44,XY,-5q12-5qter,-17,-21,-22	Abnormal
	38	17	44,XY,-9,-16	Abnormal
	38	19	46,XX	Normal
	38	22	47,XY,+8	Abnormal
95	38	1	46,XY,+21,-22	Abnormal (mosaic)
	38	2	46,XX	Normal
	38	3	45,XX,-7p,-11	Abnormal (mosaic)
	38	4	46,XY	Normal
	38	5	44,XX,-11,-14	Abnormal (mosaic for 11)
96	39	1	47,XY,-4,+21,+22	Abnormal
	39	2	47,XY,-4q(entire arm),+22	Abnormal
	39	4	46,XX	Normal
	39	6	46,XX	Normal
	39	11	No result	Undiagnosed
	39	12	45,XX,-22	Abnormal
	39	13	46,XX	Normal
	39	14	46,XY	Normal
97	39	2	46,XX	Normal
	39	6	46,XX	Normal
	39	7	47,XY,+22	Abnormal
	39	10	47,XY,+13	Abnormal
	39	12	45,XY,-20	Abnormal
	39	14	46,XY,-14,+22	Abnormal
	39	15	47,XX,+16	Abnormal
	39	21	47,XX,+16	Abnormal
	39	22	46,XX	Normal
	39	23	44,XX,-9q33-qter,-15,-16	Abnormal
	39	24	45,XY,-11,-21,+22	Abnormal
	39	25	47,XY,+22	Abnormal
	39	26	47,XY,+14	Abnormal
	39	27	47,XX,+15	Abnormal

	39	28	46,XX	Normal
	39	29	44,XY,-4,-13	Abnormal
	39	30	46,XY	Normal
98	39	1	47,XY,+15	Abnormal
	39	2	45,X0	Abnormal
	39	5	47,XY,+8	Abnormal
	39	7	45,XX,-4	Abnormal
	39	10	46,XY,-4,+19	Abnormal
	39	14	46,XX	Normal
99	39	5	46,Y0,+22	Abnormal
	39	6	45,XX,-21	Abnormal
	39	7	47,XY,+16	Abnormal
	39	8	47,XYY	Abnormal
	39	9	46,XY	Normal
	39	10	45,XX,-8	Abnormal
	39	11	46,XY	Normal
	39	13	46,XY,+6,-16	Abnormal
100	39	4	46,XX	Normal
	39	6	46,XX	Normal
	39	8	46,XX	Normal
	39	11	46,XX	Normal
	39	14	45,XY,-17	Abnormal
	39	16	46,XX	Normal
	39	19	46,XX	Normal
	39	20	45,XX,-21	Abnormal
101	39	2	46,XX,+16,-18	Abnormal
	39	4	49,XY,+5,+7,+11,-16,+22	Abnormal
	39	7	46,XY	Normal
102	39	1	47,XY,+18	Abnormal
103	39	1	No result	Undiagnosed
	39	2	44,X0,-20	Abnormal
104	39	3	46,XY	Normal
105	39	1	45,XX,-2	Abnormal
106	39	1	48,XY,+7,+10,+15,-16	Abnormal

	39	9	44,XY,-3,-16	Abnormal
107	40	1	46,XY	Normal
	40	2	47,XY,+15	Abnormal
	40	3	46,XY	Normal
	40	4	45,XY,-22	Abnormal
	40	5	45,XX,-15	Abnormal
	40	6	47,XXY	Abnormal
108	40	2	44,X,-7,-21	Abnormal
	40	3	45,XX,-15	Abnormal
	40	6	46,XX	Normal
	40	9	46,XX	Normal
	40	11	46,XX,-Xp22.3-11.23	Abnormal
	40	12	46,XX	Normal
	40	13	46,XY	Normal
	40	14	45,XX,-15	Abnormal
109	40	1	No result	Undiagnosed
	40	2	46,XX	Normal
	40	3	No result	Undiagnosed
	40	4	No result	Undiagnosed
	40	5	46,XY	Normal
	40	6	No result	Undiagnosed
	40	8	46,XY,+20,-21	Abnormal
	40	16	46,XX	Normal
	40	22	46,XX	Normal
	40	24	47,XY,+22	Abnormal
110	40	4	45,XX,-3	Abnormal
	40	5	46,XY	Normal
	40	6	46,XX	Normal
	40	8	45,XX,-15	Abnormal
	40	10	46,XY,-21,+22	Abnormal
	40	14	46,XY,-15,+17	Abnormal
	40	16	48,XX,+19,+22	Abnormal
	40	17	45,XX,-12	Abnormal
	40	18	47,XX,+16	Abnormal

	40	19	46,XY	Normal
	40	20	46,XX	Normal
	40	21	47,XY,-15,+16,+19	Abnormal
	40	22	46,XX	Normal
	40	23	46,XX,+Xp22.-p11	
	40	24	47,XY,+16	Abnormal
111	40	1	45,XY,-22	Abnormal
	40	3	SG failure/ No result	Undiagnosed
	40	8	46,XX	Normal
	40	12	47,XY,+22	Abnormal
	40	13	46,XY	Normal
	40	15	46,XX	Normal
	40	16	46,XY,-15,+21	Abnormal
112	40	1	46,XY,-13,+21	Abnormal
	40	6	45,XY,-22	Abnormal
	40	7	46,XY	Normal
	40	10	47,XX,+9	Abnormal
	40	11	46,XX	Normal
113	40	2	46,XY	Normal
	40	10	46,XX,+7,-13	Abnormal
	40	11	47,XY,+10	Abnormal
	40	12	46,XX	Normal
	40	17	43,XX,-5,-18,-21	Abnormal
	40	19	46,XX	Normal
	40	21	44,XX,-3.-13	Abnormal
114	40	1	44,XY,-4,+7,-20	Abnormal
	40	6	44,XY,-6,-20	Abnormal
115	40	3	46,XY	Normal
	40	5	46,XY	Normal
	40	12	47,XY,+16	Abnormal
116	40	1	45,XX,-21	Abnormal
	40	2	46,XY	Normal
	40	3	46,XY	Normal
	40	4	46,XX	Normal

	40	5	47,XX,+18	Abnormal
	40	6	46,XX	Normal
	40	19	47,XX,+14	Abnormal
	40	20	No result	Undiagnosed
	40	21	45,XY,-22	Abnormal
	40	22	47,XY,+21	Abnormal
	40	23	45,XX,-7,-20,+21	Abnormal
	40	24	46,XX	Normal
	40	25	45,XY,-20	Abnormal
	40	26	No result	Undiagnosed
117	40	3	46,XX	Normal
118	40	1	46,XX	Normal
	40	5	46,XY	Normal
	40	12	46,XX,+16q11.1-q24	Abnormal
	40	16	48,XY,+15,+19	Abnormal
	40	19	45,XX,-22	Abnormal
119	40	1	46,XY	Normal
	40	2	45,XX,-19	Abnormal
120	40	1	47,XX,+15	Abnormal
	40	2	47,XX,+15	Abnormal
	40	3	43,XY,-4,-5,-22	Abnormal
	40	4	45,XX,-3pter-3p21.2,+3q21.1-3qter,-22	Abnormal
	40	5	47,XY,+16	Abnormal
	40	6	47,XX,+16	Abnormal
	40	7	47,XX,+15	Abnormal
121	40	1	47,XY,+11	Abnormal
	40	2	46,XY	Normal
	40	3	46,XX	Normal
	40	4	45,XY,-18	Abnormal
	40	5	45,X0	Abnormal
	40	6	46,X0,+12	Abnormal
122	40	1	46,XX,-4q32.1-qter,+8,-20	Abnormal
	40	2	46,XY	Normal
	40	3	45,XX,-22	Abnormal

123	40	1	46,XX	Normal
	40	2	45,XXY,-5,-12	Abnormal
	40	3	46,XX,+6q11-6q23.3	Abnormal
	40	4	46,XY	Normal
	40	5	47,XY,+4	Abnormal
	40	6	46,XX	Normal
	40	7	45,Y0,-X	Abnormal
124	40	1	46,XX	Normal
	40	4	47,XY,+11	Abnormal
	40	5	44,XX,-13,-16	Abnormal
	40	7	47,XXY	Abnormal
	40	9	No result	Undiagnosed
	40	11	46,XX	Normal
	40	12	47,XX,+6	Abnormal
	40	14	47,XX,-2q11.2-qter,+15	Abnormal
125	40	1	No result	Undiagnosed
	40	4	48,XX,+6,+13	Abnormal
	40	14	47,XX,+19p13.3_p12	Abnormal
126	40	1	47,XY,+8,_7q21.3_q36.3	Abnormal
127	41	1	46,XX	Normal
	41	2	45,XY,-15	Abnormal
	41	3	47,XY,+4,-18,+22	
	41	4	46,XX	Normal
	41	5	45,XY,-15	Abnormal
	41	6	44,XY,-16,-18	Abnormal
	41	7	45,XX,+2,+9pter-p11,-17,-22	
	41	8	46,XY	Normal
	41	9	45,XY,-10	Abnormal
	41	18	46,XX	Normal
	41	19	47,XX,+3,-9,+16	Abnormal
128	41	1	SG failure/ No result	Undiagnosed
	41	2	45,XX,-18	Abnormal
	41	3	47,XYY	Abnormal
	41	4	46,XY	Normal

	41	5	46,XY	Normal
129	41	2	46,XX	Normal
	41	4	48,XY,+16,+21	Abnormal
	41	5	46,XY,-8,+22	Abnormal
	41	7	45,XO,-4,+22	Abnormal
	41	9	46,XY	Normal
130	41	1	44,XY,+2,-7,-13,-21	Abnormal
	41	2	46,XY	Normal
	41	3	46,XY	Normal
	41	5	47,XY,+16	Abnormal
	41	7	47,XY,+22	Abnormal
	41	12	46,XY,-3p26-3p11	Abnormal
	41	13	46,XX,-14,+16	Abnormal
	41	14	44,XY,-16,-21	Abnormal
	41	15	46,XY	Normal
131	41	6	45,XY,-21	Abnormal
	41	7	46,XY	Normal
132	41	1	46,XX	Normal
	41	12	44,XX,-11,-18	Abnormal
	41	14	45,XX,-14	Abnormal
	41	15	46,XX	Normal
	41	19	44,XX,-9,-11	Abnormal
	41	20	46,XX	Normal
	41	21	47,XY,-7q11.2-q36,+18	Abnormal
133	41	3	45,XX,-15	Abnormal
	41	6	47,XX,+22	Abnormal
	41	8	46,XX	Normal
	41	11	46,XY	Normal
	41	18	46,XX	Normal
134	41	2	46,XY	Normal
	41	4	47,XXY	Abnormal
	41	5	45,XY,-18	Abnormal
	41	9	47,XX,+18	Abnormal
	41	23	No result	Undiagnosed

	41	24	46,XY	Normal
	41	27	46,XX	Normal
	41	28	46,XX	Normal
135	41	1	47,XX,+1	Abnormal
	41	2	44,XY,-14,-16,-21,+22	Abnormal
	41	4	47,XXY,-14	Abnormal
	41	21	45,XX,-16	Abnormal
	41	22	47,XX,+21	Abnormal
	41	23	46,XX,+15,-22	Abnormal
	41	24	45,XY,-16	Abnormal
136	41	4	47,XXY	Abnormal
	41	8	46,XY	Normal
	41	10	45,XX,+8,-18,-22	Abnormal
	41	11	46,XY	Normal
137	41	4	46,XY	Normal
	41	6	47,XX,+20	Abnormal
	41	8	46,XY,-5q11.1-qter,+8pter-8p11.1,-8q11-8qter,-11,+16	Abnormal
	41	9	46,XX	Normal
	41	10	45,XY,-21	Abnormal
138	41	3	46,XY,-19,+22	Abnormal
	41	7	47,XX,+22	Abnormal
139	41	4	No result	Undiagnosed
	41	6	46,XY	Normal
	41	8	45,XX,-10	Abnormal
140	41	1	47,XY,+10	Abnormal
	41	3	No result	Undiagnosed
	41	4	46,XY	Normal
141	41	1	46,XX	Normal
	41	14	46,XX,-6p	Abnormal
	41	20	45,XX,+9,-15,-16	Abnormal
142	41	1	46,XY	Normal
	41	2	47,XY,-2p,+15	Abnormal
	41	3	45,XX,-9	Abnormal
	41	4	47,XY,-4p16.3_q33,+4q33_q35.2,+8,-13	Abnormal

143	41	6	45,XX,-14	Abnormal
144	41	1	46,XX,-10q	Abnormal
	41	2	46,XX,+10p,-10q	Abnormal
	41	3	46,XX	Normal
145	41	1	48,XX,+18,+21	Abnormal
	41	2	47,XX,-3p,+9,-12,+13,-17,+19	Abnormal
	41	3	44,XX,-7,-22	Abnormal
146	42	1	46,XX,+15,-18	Abnormal
	42	2	45,XY,-22	Abnormal
147	42	1	46,XX	Normal
	42	2	49,XY,+10,+12,+21	Abnormal
	42	3	46,XX,-8,+22	Abnormal
	42	4	No result	Undiagnosed
	42	5	No result	Undiagnosed
	42	6	46,XY	Normal
	42	7	47,XX,+22	Abnormal
	42	8	45,XY,-21	Abnormal
148	42	1	46,XY	Normal
	42	6	45,XX,-2,-7,+11	Abnormal
	42	10	46,XY	Normal
	42	11	46,XY	Normal
	42	12	47,XXY	Abnormal
	42	13	45,XO	Abnormal
	42	14	48,XXYY	Abnormal
149	42	2	47,XY,+17	Abnormal
150	42	1	46,XY	Normal
	42	2	No result	Undiagnosed
	42	3	47,X0,+4,+17	Abnormal
	42	4	44,XX,-14,-15	Abnormal
	42	7	46,XX	Normal
	42	14	46,XY	Normal
	42	18	47,XX,+6	Abnormal
	42	19	44,XX,-9,-15	Abnormal
	42	20	46,XX	Normal

	42	21	No result	Undiagnosed
	42	22	46,XY,-14,+21	Abnormal
	42	23	46,XY,+16,-20	Abnormal
	42	24	No result	Undiagnosed
	42	25	No result	Undiagnosed
	42	26	47,XY,-14,+16,+20	Abnormal
	42	27	No result	Undiagnosed
151	42	2	No result	Undiagnosed
	42	8	46,XY	Normal
	42	10	46,XY	Normal
	42	11	46,XY	Normal
152	42	4	46,XY,+15,-18	Abnormal
	42	5	47,XY,+17	Abnormal
	42	9	45,XY,-6	Abnormal
	42	21	46,XY,+12,-15	Abnormal
153	42	3	46,XY	Normal
	42	9	46,XY	Normal
	42	22	44,X0,-13	Abnormal
154	42	3	46,XX	Normal
	42	5	49,XX,+3,+9,+11,-16,+21	Abnormal
	42	8	47,XX,+9,+14,-15	Abnormal
155	42	1	45,XY,+7,-18,-20q,-21	Abnormal
	42	2	47,XY,+7,+12,-13	Abnormal
	42	3	46,XY	Normal
156	42	1	46,XY	Normal
	42	2	46,XX	Normal
157	42	6	46,XY	Normal
	42	7	44,XY,-18,-21	Abnormal
	42	8	47,XX,+6	Abnormal
	42	9	47,XX,+12	Abnormal
	42	11	No result	Undiagnosed
	42	12	No result	Undiagnosed
	42	14	45,XX,-18	Abnormal
	42	18	48,XX,+8,+15	Abnormal

158	42	2	46,XX,-11,+13	Abnormal
	42	6	46,XX	Normal
	42	8	45,XY,-5,-6,-7,+8,+10,+11,+13,-14,-15,-16,-17,+18,-20,+21,+22	Abnormal
	42	9	No result	Undiagnosed
159	42	1	46,XX,+8,-18	Abnormal
160	42	9	47,X0,+9+20	Abnormal
161	43	1	46,XX	Normal
	43	6	47,XY,+15	Abnormal
	43	10	46,XY	Normal
	43	14	43,XX,-9,-11,+13,-16,-18	Abnormal
	43	20	No result	Undiagnosed
	43	21	48,XY,+1,+13	Abnormal
162	43	1	47,XY,+4,+5,+9p24-q22.1,-11,+16,-21	Abnormal
	43	9	47,XX,+1	Abnormal
	43	13	46,XY,-11,+21	Abnormal
	43	14	47,XY,+19	Abnormal
	43	16	45,XX,-1p36.3-p11,+1q11-q44,-12,-13,+14	Abnormal
	43	17	46,XO,-11,+19,+22	Abnormal
	43	18	45,XX,-8	Abnormal
	43	20	45,XY,-1,-16,+17	Abnormal
163	43	6	46,XX	Normal
	43	9	48,XX,+4,+6	Abnormal
	43	13	45,XX,+4q13.3-qter,-7,-15,+18	Abnormal
164	43	1	44,XY,-2,-15	Abnormal
	43	2	46,X0,+2,+7,+8,-9,-15,+16,+17	Abnormal
	43	3	45,XY,-18	Abnormal
	43	6	47,XY,+12	Abnormal
	43	7	45,XY,+5,-11,-15,-19,+22	Abnormal
	43	16	46,XX,+3,-12,-13,-20,+21,+22	Abnormal
165	43	1	No result	Undiagnosed
	43	4	46,XX	Normal
	43	5	No result	Undiagnosed
	43	9	45,XY,-18	Abnormal
	43	13	No result	Undiagnosed

166	43	4	49,XX,+16,+19,+20	Abnormal
	43	6	45,XX,-21	Abnormal
	43	9	45,XX,-22	Abnormal
	43	11	45,XY,+15,-18,-19	Abnormal
	43	12	46,XY,+14,-16,+18,-22	Abnormal
	43	13	47,XY,-10,+15,+22	Abnormal
167	43	3	44,XX,-11,-16	Abnormal
	43	5	45,XX,+1,-8,-18	Abnormal
	43	7	48,XY,+11,+22	Abnormal
	43	8	46,XY	Normal
	43	9	45,XY,-4	Abnormal
	43	11	46,XX,+6,-15	Abnormal
168	43	7	46,XY	Normal
	43	14	45,XX,-20	Abnormal
169	43	4	47,XY,+9,+19	Abnormal
	43	5	44,XY,-11,-21	Abnormal
	43	10	45,XX,-15,+16,-21	Abnormal
170	44	8	47,XY,+15,+17,-21	Abnormal
	44	11	46,XY	Normal
	44	13	48,XY,+11,+16	Abnormal
171	44	1	45,XY,-13	Abnormal
172	44	7	No result	Undiagnosed
	44	8	41,XX,-2,-4,+7,-8,-9,+10,-11,-13,+15,-18	Abnormal
	44	11	46,XX	Normal
	44	12	47,XX,+15	Abnormal
	44	17	46,XY,-13,-15,+17,+22	Abnormal
	44	18	46,XY	Normal
	44	21	45,XY,-18,-20,+21	Abnormal
	44	22	46,XX	Normal
	44	24	45,XX,-13	Abnormal
173	44	2	45,XX,-11,-13,+16	Abnormal
	44	5	45,XX,-20	Abnormal
	44	8	47,XY,+22	Abnormal
174	44	2	46,XX,+4,-7,+12,-22	Abnormal

175	45	1	46,XY,+16,-22	Abnormal
	45	3	47,XY,+13	Abnormal
176 (1st cycle)	46	3	46,XX,-9,+17	Abnormal
	46	4	47,XX,+15	Abnormal
	46	6	45,XX,-3,-7,-10	Abnormal
	46	7	48,XX,+12,+17	Abnormal
	46	10	45,XO	Abnormal
176 (2nd cycle)	46	11	46,XX	Normal
	46	12	No result	Undiagnosed
	46	13	No result	Undiagnosed
	46	14	45,XX,-19	Abnormal
177	47	1	47,XX,+22	Abnormal
	47	4	46,XX,+15,-22	Abnormal
	47	7	45,XX,-7q33-qter,-10	Abnormal

Table D-9: Cytogenetic results for embryos at the cleavage stage from patients with recurrent miscarriages

Patient number	Maternal age	Embryo number	CGH result	Embryo characterisation
178	31	1	46,XX	Normal
	31	2	50,YY,+3,+8,-12,+14,+15,+17,-18,-19,+20,+22,-X	Abnormal
	31	3	No result	Undiagnosed
	31	4	46,XX	Normal
179	34	1	46,XXX,+1,-5,+7,-8,-10,-14,+15,-17q,+19,-22	Abnormal
	34	2	43,XYY,-1,-2,+3,-4,+5,+6,+7,-8,-9,-10,-11,-12,-13,-14,+15,+16,+17,-18,-19,+20,-22	Abnormal
	34	3	47,XX,+13	Abnormal
	34	4	46,XX	Normal
	34	5	46,XX	Normal
	34	6	46,XX,+18p	Abnormal
	34	7	41,-2,-5,+7,-8,-10,+11,+12,-14,-15,+16,+20,-21,-X,-Y	Abnormal
	34	8	45,XY,-22	Abnormal
	34	9	45,XX,-22	Abnormal
	34	10	46,XY,+1q,+3q	Abnormal
	34	11	45,XY,+7p,-7q,-16	Abnormal
180	34	1	46,XX	Normal
	34	2	47,XX,+22	Abnormal
	34	3	No result	Undiagnosed
	34	4	45,XX,-22	Abnormal
	34	5	46,XY	Normal
	34	6	47,XX,+21	Abnormal
	34	7	46,XXYY,+1,-2,-3,-4p,-6p,+7,-11,-12,+21,-22	Abnormal
	34	8	46,YY,+1,-2,+12q,-13,-15,-18,+19,+21,+22,-X	Abnormal
	34	9	46,XY,-5p,-18p	Abnormal
	34	10	No result	Undiagnosed
	34	11	46,XX	Normal
	34	12	45,XX,-16	Abnormal
	34	13	46,XYY,+1,+2,+3,-5q,-6,+8,-9,-10,-11,-12,-13,-15,+16,-18,-19,+20,+21,+22	Abnormal
	34	14	No result	Undiagnosed

	34	15	52,YY,+1,+2,+6,+9,-13,+14,-16,+17,+18,-20,+21,+22,-X	Abnormal
	34	16	46,XX	Normal
181	34	1	46,XX	Normal
	34	2	46,XY	Normal
	34	3	50,XX,+4,+12,+16,+20	Abnormal
182	34	1	47,XY,+17	Abnormal
	34	2	46,XX	Normal
	34	3	46,XY	Normal
183	35	1	47,XX,+22	Abnormal
	35	2	47,XY,+22	Abnormal
	35	3	46,XX	Normal
	35	4	48,XY,+11,+22	Abnormal
	35	5	46,XX	Normal
	35	6	45,XX,-19	Abnormal
184	35	1	46,XY	Normal
	35	4	46,XX	Normal
	35	9	46,XX	Normal
	35	10	40,XX,+4,+7,-12,-13,-14,-15,-16,-17,-18,-22	Abnormal
185	35	1	45,X0	Abnormal
	35	2	69,XXY	Abnormal
	35	3	45,XY,-2,-4,-5,+10,-12,+13,+20	Abnormal
	35	4	No result	Undiagnosed
	35	5	46,XX	Normal
	35	6	46,XY	Normal
	35	7	46,XY	Normal
	35	8	No result	Undiagnosed
186	36	1	45,XX,-8,+13,-22	Abnormal
	36	2	46,XY,-19p	Abnormal
	36	3	45,XX,-19	Abnormal
	36	6	46,XX,+6p,-6q	Abnormal
	36	7	45,XY,-14,-16q	Abnormal
	36	9	46,XY	Normal
	36	13	46,X0,-12,+13,+21	Abnormal
	36	14	46,XY	Normal

	36	15	46,XX	Normal
	36	17	48,XXY,+22	Abnormal
187	37	1	48,XX,+10,+14	Abnormal
	37	2	68,XXY,-15	Abnormal
188	38	1	46,XX,-5,-7,+8,+22	Abnormal
189	38	1	49,XX,-4,+5,+8,+10,+17,+19,-20	Abnormal
	38	2	46,XX	Normal
	38	3	46,XX	Normal
	38	4	45,XY,-22	Abnormal
	38	5	46,XY	Normal
	38	6	68,XXY,-10 (triploid)	Abnormal
	38	7	45,XY,-11	Abnormal
	38	8	No result	Undiagnosed
	38	9	46,XY,+15,-22	Abnormal
	38	10	46,XY	Normal
	38	11	46,XY,-3p26.3-q23	Abnormal
	38	12	38,XX,-4,-10,-11,-13,-15,-16,-19,-20	Abnormal
	38	13	45,XY,-13,-20q	Abnormal
	38	14	45,XY,-6	Abnormal
190	38	1	45,X0	Abnormal
	38	2	44,X0,-17	Abnormal
	38	3	46,XY	Normal
191	38	1	69,XXY, triploid	Abnormal
	38	2	49,XXY,-1,+2,-3,+5,+6,-8,+10,+11,-14,+15,+16,+18,-20,-22	Abnormal
	38	4	46,XX	Normal
	38	5	69,XXY, triploid	Abnormal
	38	7	70,XXY,+22, triploid	Abnormal
	38	8	46,XX	Normal
	38	9	47,XX,+15,+19p	Abnormal
	38	10	47,XX,+3,+4,+7,-8,-18,+19,-21	Abnormal
	38	12	45,XY,-2	Abnormal
	38	13	47,XY,+16	Abnormal
	38	14	48,XY,+2,+19	Abnormal
	38	15	48,XX,+8,+14,+19p	Abnormal

	38	18	47,XY,+1	Abnormal
192	39	1	45,XY,-2q,-9	Abnormal
	39	2	47,XX,+19	Abnormal
	39	3	47,XX,+19	Abnormal
	39	4	46,XX,+19,-21	Abnormal
	39	5	69,XXY	Abnormal
193	39	1	No result	Undiagnosed
	39	2	46,XY	Normal
	39	3	No result	Undiagnosed
	39	4	46,XY,+4,-15	Abnormal
	39	5	46,XY	Normal
	39	6	47,XY,-5,+16,+22	Abnormal
	39	7	43,XX,-1,-2,-14	Abnormal
	39	8	45,XX,-18	Abnormal
194	40	1	46,XY	Normal
	40	2	47,XY,+17	Abnormal
	40	3	46,XY	Normal
	40	4	46,XY	Normal
	40	5	No result	Undiagnosed
	40	6	47,XX,-3,+5,+6,-7,-9,+19q,+20,+21	Abnormal
	40	7	46,XY	Normal
195	42	1	47,XX,+22	Abnormal
	42	2	47,X0,+1,-2,-3,+5,+6,+7,-8,-10,+11,+12,-13,+14,-15,+16q,-17,+18,+20	Abnormal
	42	3	44,XY,-16,-22	Abnormal
	42	4	44,XX,-16,-21	Abnormal
196	42	1	46,XY	Normal
	42	2	41,XX.-1.-3.+6,-8,-9,-14,-15,+17,-18	Abnormal
	42	3	44,XY,-20,-21	Abnormal
	42	4	46,XX	Normal
	42	5	46,XX,-12,+13	Abnormal
	42	6	47,XX,+15	Abnormal
	42	7	45,XY,-10	Abnormal
	42	8	45,XY,-22	Abnormal

	42	9	50,X ,+4,+11,+14,+18	Abnormal
	42	10	46,XX	Normal
	42	11	44,XX,-13,-16	Abnormal
197	43	1	42,XX,-5,-15,-18,-22	Abnormal
	43	2	48,XX,+11,+15	Abnormal
	43	3	44,XY,-6,-10	Abnormal
	43	4	48,XY,+21,+22	Abnormal
198	43	1	46,XY,+11,-16	Abnormal
	43	2	No result	Undiagnosed
	43	3	No result	Undiagnosed
	43	4	48,XX,+8,+15	Abnormal
	43	5	48,XY,-5,+8,+10,+15	Abnormal
	43	6	No result	Undiagnosed
	43	7	47,XY,-3,+11,-15,+21,+22	Abnormal
199	43	1	No result	Undiagnosed
	43	2	46,XX	Normal
	43	3	46,XX,+1q	Abnormal
	43	4	47,XY,+9,-17,+22	Abnormal
	43	5	47,XY,+15,-16,+21	Abnormal
	43	6	48,XX,+2,+21	Abnormal
	43	7	No result	Undiagnosed
	43	8	49,XX,+1,+13,-15,+21,+22	Abnormal
200	45	1	50,XX,+5,-9,+11,+13,+19,+22	Abnormal
	45	2	45,XY,+12,-16,-21	Abnormal
	45	3	45,XX,-16	Abnormal
	45	4	46,XX	Normal
	45	5	42,XY,-3,-4,-17,-18,+19,-20	Abnormal
	45	6	49,XY,+1,-2,-3,+5,-6,+7,+8,-9,+10p,-11,+12,+14,+15,part16,+17,+18,+19q,-20,+21,-22	Abnormal
	45	7	46,X,-6,-9p,+21,-Xq	Abnormal
	45	8	47,XY,+13,-14,+19	Abnormal

Table D-10: Cytogenetic results for embryos at the blastocyst stage from patients with recurrent miscarriages

Patient number	Maternal age	Embryo number	CGH result	Embryo characterisation
201	30	8	46,XY	Normal
	30	12	47,XY,+18	Abnormal
	30	20	No result	Undiagnosed
	30	22	47,XY,+4	Abnormal
202	30	1	46,XX	Normal
	30	2	46,XX	Normal
	30	3	46,XY	Normal
	30	6	46,XX	Normal
	30	7	46,XY	Normal
	30	8	46,XY	Normal
	30	9	46,XY	Normal
	30	10	46,XY,+1q12-qter	Abnormal
203 (1st)	30	1	46,XX	Normal
	30	2	46,XY	Normal
	30	3	45,XX,-19	Abnormal
	30	4	46,XY,-18,+22	Abnormal
	30	5	40,XY,-2,+3,+4,+5,-6,-7,+9,-10,+11,-12,+13,+15,-17,+19,-20	Abnormal
	30	6	46,XY	Normal
203 (2nd)	30	2_1	46,XY	Normal
	30	2_2	45,X0	Abnormal
	30	2_3	45,XX,-5	Abnormal
	30	2_4	46,XX	Normal
	30	2_5	46,XY	Normal
	30	2_6	46,XX	Normal
	30	2_7	46,XY	Normal
204	30	1	46,XX	Normal
	30	2	45,XY,-2	Abnormal
	30	3	45,XX,-15	Abnormal
	30	4	46,XX	Normal
	30	5	46,XX	Normal

	30	6	46,XY	Normal
	30	7	46,XX	Normal
	30	8	45,XX,-21	Abnormal
	30	9	46,XY	Normal
205	31	1	45,XX,-15	Abnormal
	31	2	46,XX	Normal
	31	5	45,XX,-4	Abnormal
	31	8	46,XX	Normal
	31	13	46,XY	Normal
	31	14	46,XX,-13,+15	Abnormal
	31	15	No result	Undiagnosed
	31	17	46,XX	Normal
	31	19	46,XX	Normal
	31	20	47,XY,+2	Abnormal
206	31	1	46,XX	Normal
	31	2	46,XX	Normal
	31	3	46,XY	Normal
	31	4	46,XX	Normal
	31	5	46,XY	Normal
	31	6	47,XX,+22	Abnormal
	31	7	46,XX	Normal
	31	8	45,X0	Abnormal
	31	9	46,XX	Normal
	31	10	46,XX	Normal
	31	11	45,XY,-16	Abnormal
207	31	1	45,XX,-3q13.31_3q29,-19	Abnormal
	31	2	No result	Undiagnosed
	31	3	46,XX	Normal
	31	4	No result	Undiagnosed
	31	5	46,XY	Normal
	31	6	46,XY	Normal
	31	7	46,XX	Normal
	31	8	46,XY	Normal
208	32	1	46,XX,+15,-16	Abnormal

	32	2	47,XY,+22	Abnormal
	32	3	48,+10,+17	Abnormal
	32	4	47,XY,+22	Abnormal
	32	5	47,XX,+7	Abnormal
	32	6	46,XX	Normal
	32	7	47,XY,+15	Abnormal
	32	8	46,XX,+10pter-q22.3,-10q23.1-qter	Abnormal
	32	9	46,XY	Normal
	32	10	45,XX,-6	Abnormal
	32	11	46,XX	Normal
209	32	1	47,XY,+22	Abnormal
	32	2	No result	Undiagnosed
	32	3	45,XY,-6q24-qter	Abnormal
	32	4	46,XY	Normal
	32	5	46,XY	Normal
	32	6	46,XX	Normal
210	33	2	45,XY,-8	Abnormal
	33	3	45,XY,-2	Abnormal
	33	6	45,XY,-16	Abnormal
	33	8	45,XX,-1	Abnormal
	33	11	46,XY	Normal
	33	12	46,XX	Normal
	33	14	46,XY,-4p16-p11,+4q11-q35	Abnormal
	33	16	47,XX,-3q11.2-q29,+15	Abnormal
	33	17	46,XY	Normal
	33	19	48,XX,+7,+22	Abnormal
	33	21	48,XX,+8,+10	Abnormal
211	33	1	46,XY	Normal
	33	2	46,XY	Normal
	33	3	46,XX	Normal
	33	4	46,XY	Normal
	33	5	46,XX	Normal
	33	7	46,XY	Normal
212	34	1	SG failure/ No result	Undiagnosed

	34	2	SG failure/ No result	Undiagnosed
	34	3	SG failure/ No result	Undiagnosed
	34	5	46,XX,-2q11.2-q37.3	Abnormal
	34	6	46,XY,-15,+19	Abnormal
	34	7	45,XO	Abnormal
	34	8	47,XY,+16	Abnormal
	34	12	47,XX,+8	Abnormal
	34	13	46,XY	Normal
	34	14	46,XY	Normal
	34	17	47,XX,+19	Abnormal
	34	19	46,XY,-7q11.2-q36	Abnormal
	34	21	46,XX	Normal
	34	22	46,XY	Normal
	34	23	46,XX	Normal
213	34	3	45,XX,-16	Abnormal
	34	4	46,XX	Normal
	34	7	No result	Undiagnosed
	34	15	46,XX	Normal
214	34	1	46,Y,+9q22.2-qter,+15,-X	Abnormal
	34	2	47,XY,+16	Abnormal
	34	3	45,X0	Abnormal
	34	5	46,XX	Normal
	34	6	46,XX	Normal
215	35	2	47,XX,+8	Abnormal
	35	3	47,XY,+16	Abnormal
	35	4	45,XY,-22	Abnormal
	35	5	45,XY,-7	Abnormal
	35	6	45,Y,+15,-X	Abnormal
	35	8	47,XY,+22	Abnormal
	35	9	45,XY,-12	Abnormal
	35	12	46,XX	Normal
	35	14	46,XX	Normal
	35	16	44,XY,+1,-13,-16	Abnormal
	35	20	47,XX,+17	Abnormal

	35	21	46,XX	Normal
	35	22	46,XX	Normal
	35	23	47,XY,-4q12-qter,+21	Abnormal
	35	24	46,XX,-8,+22	Abnormal
	35	25	46,XX	Normal
	35	26	46,XX	Normal
	35	27	46,XX	Normal
	35	28	45,XY,-8	Abnormal
	35	29	46,XX,-4,+22	Abnormal
	35	30	46,XX	Normal
	35	31	46,XY,-8pter-8p11.1	Abnormal
	35	32	46,XX,+8,-19	Abnormal
	35	33	46,XY	Normal
	35	34	46,XY	Normal
	35	35	45,XY,-14	Abnormal
216	35	1	46,XY	Normal
	35	2	47,XX,+13	Abnormal
	35	3	46,XY	Normal
	35	4	47,XY,+16	Abnormal
	35	5	46,XY	Normal
217	35	1	46,XY	Normal
	35	2	46,XY	Normal
	35	3	46,XY	Normal
	35	4	45,XX,-18	Abnormal
	35	5	45,XX,-16	Abnormal
	35	6	No result	Undiagnosed
218	36	1	45,Y	Abnormal
	36	2	46,XY	Normal
	36	3	46,XX	Normal
	36	4	46,XY,-2p,+2q,+7,+20,+21	Abnormal
	36	5	No result	Undiagnosed
	36	6	No result	Undiagnosed
	36	7	46,XX	Normal
	36	8	46,XX	Normal

	36	9	47,XXY,-3,+4,+5,-6,-7,+10,-13,+14,+15,-17,+18	Abnormal
219	36	1	47,XY,+4	Abnormal
	36	3	46,XX	Normal
	36	4	46,XX	Normal
	36	5	47,XX,+15	Abnormal
220	36	1	45,XX,-16	Abnormal
	36	2	45,XX,-16	Abnormal
	36	3	46,XY	Normal
	36	4	46,XX,-Xp22.2-p21.1	Abnormal
	36	5	46,XX	Normal
	36	10	46,XX	Normal
	36	11	46,XY	Normal
	36	12	45,Y0,-X	Abnormal
	36	13	46,XX	Normal
	36	14	45,XX,-4	Abnormal
	36	15	46,XY	Normal
	36	22	44,XX,-16,-22	Abnormal
	36	23	45,XY,-22	Abnormal
	36	24	45,XXY,-16,-21	Abnormal
	36	25	46,XY,-1,+22	Abnormal
221	36	1	46,XX	Normal
	36	2	45,XY,-22	Abnormal
	36	3	46,XY	Normal
	36	4	46,XX	Normal
	36	5	46,XX	Normal
	36	6	No result	Undiagnosed
222	37	2	45,XO	Abnormal
	37	5	46,XX	Normal
	37	7	45,XX,-15	Abnormal
	37	12	46,XX	Normal
	37	16	46,XY	Normal
223	38	1	46,XX	Normal
	38	4	45,XY,-2	Abnormal
	38	5	46,XY	Normal

	38	6	45,XO	Abnormal
	38	12	46,XY	Normal
	38	14	45,XY,-22	Abnormal
	38	15	SG failure/ No result	Undiagnosed
	38	16	SG failure/ No result	Undiagnosed
	38	21	SG failure/ No result	Undiagnosed
	38	22	SG failure/ No result	Undiagnosed
	38	23	46,XX	Normal
	38	24	44,XX,-18,-22	Abnormal
224	38	1	49,XY,+3,+21,+22	Abnormal
	38	2	46,XX,-2p25.3_2p16.3,-8q11.1_q24.3,-10p15.3_p11.21,-12p13.3_12p12.2,+14q23.3_q32.3,-22q13.2_q13.33	Abnormal
	38	3	47,XX,+6	Abnormal
225	38	1	46,XX	Normal
	38	2	46,XX,+1q31.2_q44,-2q22.3_q37.3	Abnormal
	38	3	42,XX,-1q31.2_q44,+2q22.3_q37.3,-3,-4,-17,-22	Abnormal
	38	4	46,XY	Normal
	38	5	46,XX	Normal
	38	6	46,XX,-21,+22	Abnormal
226	38	4	46,XX	Normal
	38	8	46,XX	Normal
	38	9	47,XY,+10	Abnormal
227	39	4	46,XX	Normal
	39	5	47,XY,+17	Abnormal
	39	9	46,XX	Normal
	39	11	47,XX,-11,+12,+22	Abnormal
	39	12	46,XY	Normal
	39	13	46,XY	Normal
	39	14	46,XX	Normal
	39	15	45,XX,-18	Abnormal
	39	16	44,X0,-5	Abnormal
	39	17	46,XY	Normal
	39	18	46,XY	Normal
	39	19	45,XX,-16	Abnormal

	39	20	46,XX,-9,+22	Abnormal
	39	21	47,XY,+2	Abnormal
228	40	1	46,XX,+13,-16	Abnormal
	40	2	47,XY,+16,+20p13-p11.1	Abnormal
	40	3	44,XY,-5,+16,-21	Abnormal
	40	4	46,XX,-2,+22	Abnormal
	40	5	45,XX,-16	Abnormal
	40	6	47,XY,+22	Abnormal
	40	7	47,XX,+22	Abnormal
	40	8	46,XY	Normal
	40	9	47,XY,+9,+22	Abnormal
229	40	1	44,XX,-13,-21	Abnormal
	40	2	47,XXY	Abnormal
	40	3	46,XY	Normal
	40	4	No result	Undiagnosed
	40	5	45,XX,-21	Abnormal
	40	6	47,XXY	Abnormal
230	40	1	47,XY,+16	Abnormal
	40	2	46,XY	Normal
	40	4	47,XX,+20	Abnormal
	40	7	44,XY,-21,-22	Abnormal
	40	9	No result	Undiagnosed
	40	10	46,XY	Normal
	40	11	44,XX,-21,-22	Abnormal
	40	12	46,XY	Normal
231	40	1	48,XX,+8,+11	Abnormal
	40	2	46,XX	Normal
	40	3	46,XY	Normal
	40	4	47,XY,+15	Abnormal
	40	5	47,XY,+22	Abnormal
	40	6	47,XY,+9	Abnormal
	40	7	46,XY	Normal
	40	8	46,XX	Normal
232	40	1	46,XX,+6q	Abnormal

	40	2	47,XY,+1,-2,+20	Abnormal
233	41	2	No result	Undiagnosed
	41	5	46,XX,-16,+22	Abnormal
	41	6	46,XY,+1q31-q44	Abnormal
	41	7	47,XY,+19	Abnormal
	41	10	46,XY,+10,+19,-20,-21	Abnormal
	41	11	46,XX	Normal
	41	12	45,XX,-4	Abnormal
	41	13	47,XY,+22	Abnormal
234	41	6	46,X0,+22	Abnormal
	41	8	46,XY	Normal
	41	10	48,Y,+1,+12,+17	Abnormal
	41	11	47,XX,+21	Abnormal
235	41	5	46,XX,-10,+16	Abnormal
	41	6	48,XX,-3q36.1-q29,+10,+20	Abnormal
	41	7	47,XY,+13	Abnormal
	41	8	45,XX,-13	Abnormal
	41	9	46,XX	Normal
	41	10	46,XX	Normal
	41	11	46,XX	Normal
	41	13	45,XY,-1	Abnormal
236	41	41	45,XX,-21	Abnormal
237	42	1	45,XO	Abnormal
	42	2	46,XX	Normal
	42	3	No result	Undiagnosed
	42	4	46,XY	Normal
	42	5	47,XX,+8	Abnormal
	42	6	47,XX,+19	Abnormal
	42	9	47,XY,+22	Abnormal
238	42	1	68,XXY,-22	Abnormal
	42	2	44,XY,-5,-21	Abnormal
	42	3	45,XX,-12	Abnormal
	42	4	46,XY	Normal
	42	5	46,XX	Abnormal

	42	6	47,XY,+9,-21,+22	Abnormal
	42	7	43,XX,-11,-15,-22	Abnormal
	42	8	46,XX	Normal
239	43	1	45,XY,-4,+16,+22	Abnormal
	43	3	No result	Undiagnosed
	43	4	46,XY	Normal
240	43	1	46,XY	Normal
241	43	1	46,XY,+15,-22	Abnormal
	43	2	46,XX,-4q	Abnormal
	43	3	44,X,-1,-14,-16,+19	Abnormal
	43	4	47,XY,-2,+7,-11,+18,+22	Abnormal
	43	5	46,XX,-2,+22	Abnormal

Table D-11: Cytogenetic results for embryos at the cleavage stage from patients with repeated IVF failure

Patient number	Maternal age	Embryo number	CGH result	Embryo characterisation
242	27	2	No result	Undiagnosed
	27	3	43,XY,-5,+7,-9,-10,-11,+12,-13,+14,-15,+17,+18,-20,-22	Abnormal
	27	4	46,XX	Normal
	27	5	46,XY,+16q	Abnormal
	27	6	45,X0	Abnormal
	27	7	49,XXY,+6,+13,+19p	Abnormal
	27	8	46,XX,+19p13.31_13.43	Abnormal
243	30	1	49,XX,-1,-2,+3,-11,+12,+16,+19,+20,+21	Abnormal
	30	2	44,XX,-8,-19	Abnormal
	30	3	47,XY,-16q,+19	Abnormal
	30	4	46,XY,+Xp	Abnormal
	30	5	41,X0,-1,-4,-18,-22	Abnormal
	30	6	49,XXYY,+1,+4,-13,+18,-20	Abnormal
	30	7	46,XY	Normal
	30	8	46,XX	Normal
	30	9	45,XY,-9	Abnormal
	30	10	46,XX,+13,-19	Abnormal
	30	11	45,XY,-16	Abnormal
	30	12	46,XX	Normal
	30	13	No result	Undiagnosed
244	31	1	46,XX	Normal
	31	2	46,XY,-3q	Abnormal
	31	3	51,XX,-1p,+3,+11,+16,+17,+21	Abnormal
	31	4	46,XX	Normal
	31	5	46,XY,-3,+9	Abnormal
	31	6	46,XY	Normal
	31	7	47,XX,+1,+2,+8,-12,-18	Abnormal
245	34	1	47,XX,+22	Abnormal
	34	2	46,XX	Normal
	34	3	45,XX,-21	Abnormal

	34	4	46,XY	Normal
	34	5	46,XX,+1p/q,-3,-5(x2),+6,+7,+8,+10,+12,-13,-14,-18,+19,-20	Abnormal
	34	6	46,XX	Normal
246	34	1	48,XY,-9p,+14,+20	Abnormal
	34	2	46,X0,-9,+16,+22	Abnormal
	34	3	43,X0,-20,-22	Abnormal
	34	4	48,XX,+7,+19	Abnormal
	34	5	45,XY,-8	Abnormal
	34	6	45,XX,-4	Abnormal
247	35	2	46,XX	Normal
	35	3	48,XY,+14,+22	Abnormal
	35	6	46,XX,-7q	Abnormal
	35	11	47,XX,+13	Abnormal
248	35	1	47,XX,+16	Abnormal
	35	2	45,XX,-22	Abnormal
	35	3	48,XX,+16,+22	Abnormal
	35	4	46,XX	Normal
249	36	1	No result	Undiagnosed
	36	2	46,XX,-2,+3,+11,-12	Abnormal
250 (1st)	37	2	50,XY,+1,+7,+12,+18	Abnormal
	37	3	45,XY,+7q,-10,-22	Abnormal
	37	4	47,XY,+8	Abnormal
	37	5	44,XY,-1,-3,-7,+20	Abnormal
	37	8	No result	Undiagnosed
	37	11	46,XX,+12p,-12q	Abnormal
	37	12	44,XX,-19,-22	Abnormal
250 (2nd)	37	2_1	46,XY,-18q	Abnormal
	37	2_2	46,XY	Normal
	37	2_3	47,X0,+1,+4,-5,+10,+11,-12,+13,-14,+15,-16,-19,+20,-21,+22	Abnormal
	37	2_4	47,XX,+17	Abnormal
	37	2_5	69,XXY	Abnormal/triploid
	37	2_6	43,Y0,-5,-11	Abnormal
	37	2_7	46,XX,+16,-21	Abnormal
	37	2_8	46,XY,-6q	Abnormal

251	37	1	No result	Undiagnosed
	37	2	45,XX,-21	Abnormal
	37	3	No result	Undiagnosed
252	38	1	47,XX,+22	Abnormal
	38	2	47,XY,-1,+10,+11	Abnormal
	38	3	46,XY	Normal
	38	4	46,XX	Normal
	38	6	49,XY,+7,+8,+13	Abnormal
	38	8	42,,XY,-7,-8,-14,-15	Abnormal
253	39	1	46,XX	Normal
	39	3	45,XX,-22	Abnormal
	39	4	46,XY,+10p,-14,+17	Abnormal
	39	7	45,XX,-21	Abnormal
	39	9	46,XX	Normal
	39	10	46,XY	Normal
	39	13	46,XX	Normal
254	39	1	45,XY,+8p,-14	Abnormal
	39	2	47,XX,-1p,+22	Abnormal
	39	3	46,XY,-2p,-9q	Abnormal
	39	4	45,XXY,-6,-11,+19,-20,-21,+22	Abnormal
	39	5	46,XY	Normal
	39	6	46,XY,-15,+17	Abnormal
	39	7	48,XX,+2,+16	Abnormal
	39	8	46,XY,+11,-22	Abnormal
	39	9	46,XX	Normal
255	42	1	43,X0,+10,-11,-18,-19	Abnormal
	42	2	48,Y0,+1,-2,-3,+6,+10,+12,+13,-14,-16,-17,+19,-20,+21,-X	Abnormal
	42	4	45,Y0,+1,-2,-3,+6,-7,+10,+12,+13,-14,-15,-16,+17,+19,-21,-X	Abnormal
	42	5	45,XX,-20	Abnormal
	42	6	47,XY,-8,+13,+19	Abnormal
	42	7	46,XY,-16,+19	Abnormal
256	42	1	No result	Undiagnosed
	42	2	47,XX,+2	Abnormal
	42	3	47,XY,-9q,+14,+21,-22	Abnormal

	42	4	48,XXX,+1,+2,-4,+6,+7,+9,-10,-11p,-12,-15,+19,-21	Abnormal
	42	5	43,XY,-13,-15,-16,-19,+22	Abnormal
257	42	1	46,XX	Normal
	42	2	45,XX,+1,+2,-3,+4,-5,+6,+7,-9,+12,-13,-14,+15,-16,+17,-18,-19,-20,-21,+22	Abnormal
	42	3	45,XY,-1q,-5q,-9,+16,-22	Abnormal
	42	4	47,XY,+13q,+15,-20,+22	Abnormal
	42	5	45,XX,-21	Abnormal
	42	6	44,XX,-1,+4,+7,+8,-11,+12,+13,-14,+17,+19,-21	Abnormal
	42	7	44,XY,-18,-21	Abnormal
	42	8	No result	Undiagnosed
	42	9	47,XX,+12,-17,+18	Abnormal
	42	10	50.XY,-1q,+3,+10,+15,+16,-17,+21,+22	Abnormal
	42	11	45,XY,-16	Abnormal
	42	12	50,XXX,-1,+2,+3,-5,-6,+7,-10,+16,+19,+21	Abnormal
	42	13	46,XY,-2,+3,+7,+12,-16,-17	Abnormal
	42	14	No result	Undiagnosed
	42	15	46,X0,+10	Abnormal
	42	16	No result	Undiagnosed
	42	17	No result	Undiagnosed
	42	18	44,XY,+10,-12,-16,-17	Abnormal
	42	19	46,XXX,+1,-3,-5,-11,-13,+15,-17,+18,+X	Abnormal
	42	20	47,XX,+22	Abnormal
	42	21	42,Y0,-7,+11,-15,+17,-18,-19,-20,-X	Abnormal
	42	22	46,XY	Normal
258	42	1	47,XY,+14	Abnormal
	42	2	No result	Undiagnosed
	42	3	48,XX,+12,+13	Abnormal
	42	5	47,XXX,-1,-2,-4,+6,+7,+11,+12,-13,+19,-20	Abnormal
	42	6	69,XXY,+1,-9 (triploid)	Abnormal
	42	7	50,XXX,+2,+3,+4,-5,+7,+11,-16,-19,+22	Abnormal
	42	8	44,XY,-1,-8	Abnormal
	42	9	43,XY,-1,+2,-7,-9,-13	Abnormal
	42	10	No result	Undiagnosed
	42	11	47,XX,+16	Abnormal

	42	12	47,XX,-2,+13,+22	Abnormal
	42	13	46,XX,+15,-18	Abnormal
	42	14	No result	Undiagnosed
	42	15	46,XY,+8,+11,-14,-18	Abnormal
	42	16	45,XX,+6,-7,-9,-10,-14,+15,+17,+19,-20	Abnormal
259	44	1	45,XX,-2,-3.-4.+5,-7,+9,+12,+13,+14,-15,-16,-17,-18,-19,+20,+21,-22	Abnormal
	44	2	39,X0,-5,-11,-17,-18,-21,-22	Abnormal
	44	3	46,XX	Normal
	44	4	46,XX,-3q	Abnormal
	44	5	44,XX,-4,-10,-12p,+12q	Abnormal
	44	6	41,-1,-4,+5,-6,+7,-8,-17,-19,-20,+21,-X,-Y	Abnormal
	44	7	47,XX,+16	Abnormal

Table D-12: Cytogenetic results for embryos at the blastocyst stage from patients with repeated IVF failure

Patient number	Maternal age	Embryo number	CGH result	Embryo characterisation
260	28	2	46,XY	Normal
	28	3	46,XX	Normal
	28	4	46,XY	Normal
	28	5	46,XX	Normal
	28	6	46,XX	Normal
	28	7	46,XY	Normal
	28	8	46,XX	Normal
	28	9	48,XX,+14,+20	Abnormal
261	29	1	46,XX	Normal
	29	4	46,XX	Normal
	29	5	47,XY,+16	Abnormal
	29	10	46,XY	Normal
	29	19	46,XX,+1p36.3-p11	Abnormal
	29	20	46,XX	Normal
	29	22	47,XY,+18	Abnormal
262	32	1	46,XY	Normal
	32	4	46,XY	Normal
	32	5	46,XX	Normal
	32	8	No result	Undiagnosed
	32	13	47,XX,+4	Abnormal
	32	14	47,XX,+15,+2p25.2-2q12.1	Abnormal
	32	16	46,XX	Normal
	32	17	45,XY,-16	Abnormal
	32	20	46,XX	Normal
263	32	1	No result	Undiagnosed
	32	2	46,XX	Normal
	32	3	46,XX	Normal
	32	4	46,XX	Normal
	32	5	47,XY,+15	Abnormal
	32	6	46,XX	Normal
	32	7	47,XXY	Abnormal

	32	8	45,XX,-22	Abnormal
	32	9	46,XY	Normal
	32	10	45,XY,-22	Abnormal
	32	11	46,XY	Normal
	32	13	46,XX	Normal
	32	14	47,XY,+13	Abnormal
	32	15	45,XY,-16	Abnormal
	32	16	46,XY	Normal
	32	17	45,XX,-3,-12,+15	Abnormal
	32	18	46,XX	Normal
	32	19	48,XX,+7,-11q22.1-q25,+13	Abnormal
	32	20	46,XY	Normal
264	33	2	46,XX	Normal
	33	8	46,XX	Normal
	33	11	45,XO	Abnormal
	33	14	47,XY,+1	Abnormal
	33	15	46,XY	Normal
265	34	1	47,XX,+19	Abnormal
	34	2	47,XX,+19	Abnormal
	34	3	45,XX,-12	Abnormal
	34	4	47,XX,+17	Abnormal
266	34	3	46,XX	Normal
	34	4	46,XX	Normal
	34	6	46,XX	Normal
	34	13	46,XX,-13,+16	Abnormal
	34	18	47,XXY	Abnormal
	34	19	46,XY,+16pter-p11.1	Abnormal
	34	20	46,XX	Normal
	34	21	46,XX	Normal
	34	22	46,XX	Normal
	34	23	45,XX,-11	Abnormal
	34	24	46,XY	Normal
	34	25	46,XX	Normal
	34	26	46,XY	Normal

	34	27	46,XX	Normal
	34	28	46,XX	Normal
	34	29	46,XY	Normal
267	34	1	46,XX	Normal
	34	8	No result	Undiagnosed
268	35	1	46,XY	Normal
	35	2	46,XX	Normal
	35	3	46,XX	Normal
	35	4	45,XY,-8	Abnormal
	35	5	45,X0	Abnormal
269	36	5	46,XX	Normal
	36	10	46,XX	Normal
	36	16	46,XY	Normal
	36	18	46,XX	Normal
270	36	3	45,X0	Abnormal
	36	6	47,XY,+16	Abnormal
	36	7	46,XY	Normal
	36	11	46,XY,-7q11.22-qter	Abnormal
	36	12	47,XX,+15	Abnormal
	36	17	46,XY,-5pter-5p12	Abnormal
	36	18	46,XX	Normal
	36	19	45,XY,-13	Abnormal
	36	20	45,XX,-4,+15,-21	Abnormal
	36	21	47,XY,+12	Abnormal
	36	22	47,XY,+22	Abnormal
	36	23	No result	Undiagnosed
271	36	1	47,XY,-4,+12,+22	Abnormal
	36	2	45,XX,-18,+19,-20	Abnormal
	36	7	46,XX	Normal
	36	9	46,XX,-4,+9	Abnormal
	36	10	47,XX,-4,+20,-21	Abnormal
	36	11	47,XX,+16	Abnormal
	36	13	No result	Undiagnosed
	36	17	46,XY,-6q24.1-qter	Abnormal

	36	20	46,XX	Normal
272	36	7	46,XY	Normal
	36	14	48,XX,-3,+12,+13,+18	Abnormal
	36	19	45,XX,-19	Abnormal
	36	21	47,XY,+1	Abnormal
273	36	2	46,XX	Normal
	36	3	46,XY	Normal
	36	7	45,XY,-13	Abnormal
	36	8	46,XY	Normal
	36	10	46,XY	Normal
	36	11	47,XX,+15	Abnormal
274	36	1	No result	Undiagnosed
	36	3	45,XX,-16	Abnormal
	36	4	46,XY	Normal
	36	6	45,XX,-16	Abnormal
	36	10	47,XX,+15	Abnormal
275	36	5	46,XY,-1,+21	Abnormal
	36	7	47,XX,+19	Abnormal
276	37	1	47,XY,+22	Abnormal
	37	2	43,XX,-8,-13,-18	Abnormal
	37	3	44,XX,-16,-22	Abnormal
	37	4	46,XY	Normal
	37	5	45,XY,-21	Abnormal
	37	6	48,XXYY	Abnormal
	37	7	46,XY	Normal
	37	8	47,XY,+21	Abnormal
	37	9	46,XX	Normal
	37	10	47,XX,+21	Abnormal
277	37	1	46,XY	Normal
	37	2	44,XX,-+1q21.1-q44,-21,-22	Abnormal
	37	3	46,XY	Normal
	37	4	46,XY	Normal
	37	5	45,XY,+1q21.1-q44,-2	Abnormal
	37	6	46,XY	Normal

	37	7	46,XX	Normal
	37	10	46,XY	Normal
	37	11	47,XY,+19	Abnormal
278	**37**	3	46,XY	Normal
	37	5	47,XY,+19	Abnormal
	37	12	45,XX,-4	Abnormal
	37	13	48,XX,+19,+22	Abnormal
	37	15	46,XY	Normal
279	37	3	46,XY	Normal
	37	5	47,XXY	Abnormal
	37	8	48,XX,+9,+11	Abnormal
	37	12	46,XY	Normal
	37	14	47,XY,+17	Abnormal
	37	17	44,XX,-16,-21	Abnormal
	37	19	44,XX,-19,-22	Abnormal
	37	20	46,XX	Normal
280	**37**	2	47,XX,+22	Abnormal
	37	8	46,XX	Normal
	37	11	47,XY,+15	Abnormal
	37	12	46,XY	Normal
	37	15	46,XY,-11p15.5-p11.1	Abnormal
281	**37**	2	47,XY,+16	Abnormal
	37	3	47,XY,+16	Abnormal
	37	6	47,XX,+20	Abnormal
282	38	1	47,XYY	Abnormal
	38	2	46,XY	Normal
	38	3	46,XX	Normal
	38	4	46,XY	Normal
	38	5	46,XY	Normal
	38	6	SG failure/ No result	Undiagnosed
283	38	6	46,XY	Normal
	38	7	45,XY,-21	Abnormal
	38	9	46,XY,-5p	Abnormal
	38	11	46,XY	Normal

	38	12	45,XX,-19	Abnormal
284	39	1	46,XX	Normal
	39	2	46,XX	Normal
	39	3	46,XY	Normal
	39	4	46,XY	Normal
	39	5	45,XX,-2,+14,-17	Abnormal
	39	6	47,XX,+18	Abnormal
	39	7	46,XX	Normal
	39	8	47,XX,+6,+17,-21	Abnormal
	39	9	47,XX,+16	Abnormal
	39	10	45,XY,-21	Abnormal
	39	11	44,XX,-13,-16	Abnormal
	39	12	46,XY	Normal
285	39	1	47,XY,+22	Abnormal
	39	2	46,XX,-16,+21	Abnormal
	39	3	No result	Undiagnosed
	39	6	46,XX	Normal
	39	7	46,XX	Normal
	39	9	47,XY,+6	Abnormal
	39	10	47,XY,+13	Abnormal
	39	11	46,XX	Normal
286	**40**	1	46,XY	Normal
	40	2	47,XX,+21	Abnormal
287	40	1	46,XX	Normal
	40	2	45,X0	Abnormal
	40	9	46,XY	Normal
	40	10	48,XY,+16,+22	Abnormal
	40	11	46,XY	Abnormal
288 (1st)	**40**	1	46,XY	Normal
	40	2	46,XX	Normal
	40	3	46,XX,-Xq	Abnormal
	40	4	46,XX,-16,+Xq	Abnormal
288 (2nd)	**40**	C2_1	47,XX,+8	Abnormal
	40	C2_3	47,XY,-13q,+19	Abnormal

	40	C2_4	47,XY,+16	Abnormal
289	40	1	47,XY,+12	Abnormal
	40	3	47,XX,+7p,+22	Abnormal
	40	4	46,XX	Abnormal
290	**41**	3	No result	Undiagnosed
	41	4	46,XX	Normal
	41	5	45,XX,-12	Abnormal
	41	11	46,XY	Normal
	41	12	No result	Undiagnosed
291	**41**	1	47,XY,+22	Abnormal
	41	2	46,XY,+2,-4	Abnormal
	41	3	46,XX	Normal
	41	4	48,XY,+1,+15	Abnormal
	41	5	No result	Undiagnosed
292	41	C2_1	45,XX,-14	Abnormal
	41	C2_2	47,XY,+15	Abnormal
	41	C2_3	45,XX,-19	Abnormal
293	41	1	46,X0,+18	Abnormal
	41	2	47,XX,+5,+15	Abnormal
	41	3	46,XY,+17,-22	Abnormal
	41	4	46,XY	Normal
	41	5	46,XY,+11pter-11p14,-11p13-11qter,+17,-22	Abnormal
	41	6	45,XY,-16	Abnormal
	41	7	47,XY,+15,+22	Abnormal
	41	8	46,XX	Normal
	41	9	46,XX	Normal
	41	10	45,XY,+11,-16,-22	Abnormal
	41	11	46,XX	Normal
	41	12	45,XY,-3	Abnormal
294	41	1	46,XY	Normal
	41	2	47,XY,+2	Abnormal
	41	3	44,XX,-8,-21	Abnormal
	41	4	47,XX,-11,+19,+22	Abnormal
	41	5	SG failure/ No result	Undiagnosed

	41	6	48,XX,+15,+18	Abnormal
	41	7	46,XX	Normal
	41	8	46,XY	Normal
295	**42**	4	47,XY,+22	Abnormal
	42	8	46,XX	Normal
296	42	1	47,XY,+15	Abnormal
	42	2	47,XX,+22	Abnormal
	42	3	46,XX	Normal
	42	4	46,XY,-16q11.1-q24	Abnormal
	42	5	46,XX,+4,-20	Abnormal
	42	6	45,XY,-15	Abnormal
	42	7	46,XY	Normal
	42	8	46,XX	Normal
	42	9	46,XX	Normal
	42	10	47,XX,+17	Abnormal
	42	11	45,XY,-16	Abnormal
	42	12	46,XY,-18,+22	Abnormal
	42	13	46,XX	Normal
297	42	2	46,XX,-5,+20	Abnormal
	42	5	46,XX,-1,+16	Abnormal
298	42	3	46,XY	Normal
	42	9	46,XX	Normal
	42	16	43,XX,-7,-15,-20	Abnormal
299	43	1	47,XX,+16	Abnormal
	43	2	46,XXY,-22	Abnormal
	43	3	45,XY,-14	Abnormal
300	**44**	11	No result	Undiagnosed
	44	12	44,XY,-2,-3,+10,-21	Abnormal
	44	16	48,XYY,-14,+16,+17	Abnormal
	44	19	45,XO,+16,-21	Abnormal

Appendix E

1. A list of oral presentations in international meetings:

- **Alfarawati S,** Fragouli E, Katz-Jaffe M, Schoolcraft WB, Munne S, Wells D. Comprehensive cytogenetic analysis of the blastocyst stage: the impact of aneuploidy on embryo morphology. 25th Annual meeting of the European Society of Human Reproduction and Embryology (ESHRE). 2009. Amsterdam, Netherlands.
- **Alfarawati S,** Goodall N, Gordon T, Gunner J, Daphnis D, Wells D, Fragouli E. Cytogenetic analysis of human blastocysts with the use of FISH, CGH and aCGH: scientific data and technical evaluation. 26th Annual meeting of the European Society of Human Reproduction and Embryology (ESHRE). 2010. Rome, Italy.
- **Alfarawati S,** Fragouli E, Mania A, Griffiths T, Gordon T, Daphnis D, Wells D. Comprehensive characterization of chromosome abnormalities in human blastocysts. Fertility 2011. Dublin. Ireland.
- **Alfarawati S,** Fragouli E, Konstantinidis M, Wells D. Are patients undergoing PGD for chromosome rearrangements at increased risk of aneuploidy affecting chromosomes unrelated to their rearrangement (interchromosomal effect)? *67th annual meeting of the American Society for Reproductive Medicine (ASRM).* 2011. Orlando, USA.
- **Alfarawati, S**, Poli M, Wells D, Fragouli E. How does aneuploidy affect embryo morphology and development from the cleavage to the blastocyst stage? 28th Annual meeting of the European Society of Human Reproduction and Embryology (ESHRE). 2012. Istanbul, Turkey.

  This presentation was nominated for the Fertility Society of Australia Exchange Award

A list of publications and presentations from this project:

- **Alfarawati** S, Fragouli E, Colls P, Wells D. Embryos of robertsonian translocation carriers exhibit a mitotic interchromosomal effect that enhances genetic instability during early development. PLoS Genet. 2012 Oct;8(10):e1003025.

- **Alfarawati S,** Fragouli E, Colls P, Wells D. First births after preimplantation genetic diagnosis of structural chromosome abnormalities using comparative genomic hybridization and microarray analysis. Hum Reprod. 2011 Jun;26(6):1560-74.

- **Alfarawati S,** Fragouli E, Colls P, Stevens J, Gutiérrez-Mateo C, Schoolcraft WB, Katz-Jaffe MG, Wells D. The relationship between blastocyst morphology, chromosomal abnormality, and embryo gender. Fertil Steril. 2011 Feb;95(2):520-4.

- Fragouli E, **Alfarawati S**, Daphnis DD, Goodall NN, Mania A, Griffiths T, Gordon A, Wells D. Cytogenetic analysis of human blastocysts with the use of FISH, CGH and aCGH: scientific data and technical evaluation. Hum Reprod. 2011 Feb;26(2):480-90.

- Fragouli E, **Alfarawati S,** Goodall NN, Sánchez-García JF, Colls P, Wells D. The cytogenetics of polar bodies: insights into female meiosis and the diagnosis of aneuploidy. Mol Hum Reprod. 2011 Apr 14. [Epub ahead of print].

- Wells D, **Alfarawati S,** Fragouli E. A skewed sex ratio following blastocyst culture is a consequence of embryo grading systems that prioritise male embryos for transfer. BJOG. 2011 Feb;118(3):381.

- Fragouli E, Katz-Jaffe M, **Alfarawati S**, Stevens J, Colls P, Goodall NN, Tormasi S, Gutierrez-Mateo C, Prates R, Schoolcraft WB, Munne S, Wells D. Comprehensive chromosome screening of polar bodies and blastocysts from couples experiencing repeated implantation failure. Fertil Steril. 2010 Aug;94(3):875-87.